PureExtreme®

Highest quality & performance

We guarantee that these products are free of contaminating activities.
Our stringent quality control with the most advanced tests guarantees you pure products for your experiments. ISO9001 and ISO14001 is your assurance of consistency and lot-to-lot reproducibility.
PureExtreme® Quality will provide the performance you need for your most demanding experiments.

PROTEIN ELECTROPHORESIS & ANALYSIS

Protein Electrophoresis & Analysis: Introduction

Protein Extraction (*see* p.392)

Extraction, quantification and analysis of native proteins are fundamental steps in proteomics research. However, protein extraction from *in vitro* and *in vivo* systems can pose a serious challenge due to protein heterogeneity and the resistance of the cytoplasmic and nuclear membrane to rupture. Fermentas now offers two new products for efficient protein extraction from cultured cells or tissues: **ProteoJET™ Mammalian Cell Lysis Reagent** (#K0301) for one-step total protein extraction and **ProteoJET™ Cytoplasmic and Nuclear Protein Extraction Kit** (#K0311) for simultaneous isolation of nuclear and cytoplasmic protein fractions from the same sample. Both kits use a mild detergent-based lysis step that permits extraction of native proteins suitable for common proteomics and enzymatic activity assays including immunoprecipitation, affinity purification, 1D and 2D SDS-PAGE, Western blotting and electrophoretic mobility shift assays (EMSA). Proteins extracted with these kits are also compatible with Bradford and other quantification assays.

Protein Quantification (*see* p.394)

The Bradford assay is one of the most convenient and popular protein quantification procedures as it allows fast, sensitive and accurate results. Fermentas offers a ready-to-use **Bradford Reagent** (#R1271). Protein concentration is determined by comparing the spectrophotometric absorption value of the sample to the standard curve values obtained with protein solutions of a known concentration. The accuracy of protein quantification depends on the purity of the proteins used to create the standard curve. Fermentas offers a choice of two new protein standard solution sets: **Bovine Serum Albumin Standard Set** (#R1281) and **Bovine Gamma Globulin Standard Set** (#R1291). Both are composed of highly purified proteins at seven ready-to-use concentrations, eliminating laborious dilution steps. In addition to these products, our bovine serum albumin (BSA)-based **Protein Standard Solution** (#R0882) is a universal standard for colorimetric determination of protein concentrations by various methods including Lowry and Bradford assays.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE is the most widely used method for characterization of proteins and evaluation of their molecular weights and purity. Nearly all proteins are soluble in the presence of the anionic detergent sodium dodecyl sulfate (SDS). SDS binds to proteins with high affinity which confers a negative charge to the polypeptides and relaxes higher order protein structures. Complete disruption of tertiary and quaternary protein structures is essential to achieve protein separation according to molecular weight. This is accomplished by reduction of the inter- and intra-molecular disulfide bonds of polypeptides by the addition of agents such as dithiothreitol (DTT, #R0821) or 2-mercaptoethanol and subsequent heating. Our new **DualColor™ Protein Loading Buffer Pack** (#R1011) and classical **Protein Loading Buffer Pack** (#R0891) contain all the necessary components for the preparation of protein samples for SDS-PAGE analysis (p.404). The DualColor™ Protein Loading Buffer Pack has two tracking dyes: blue and pink. The pink dye assists with monitoring protein migration during Western Blot transfers as it remains visible on the membrane following transfer from the gel.

SDS-PAGE normally is performed in **Tris-glycine-SDS** (#B46) electrophoresis buffer. However, for separation of small proteins and peptides, **Tris-tricine-SDS** (#B48) electrophoresis buffer is recommended (p.401).

Native Protein Electrophoresis

Non-denaturing electrophoresis is used to analyze proteins while preserving native structure and conformation. Under native conditions, the electrophoretic separation of proteins depends on their size, shape and charge. Native protein electrophoresis is often carried out in **Tris-glycine** (#B47) electrophoresis buffer (p.401). Protein samples used for native electrophoresis should be devoid of strong denaturants such as SDS.

Estimation of Protein Molecular Weight: Protein Ladders and Marker (*see* p.396)

Fermentas offers both **Prestained Protein Ladders and Markers** (p.396) and **Unstained Protein Ladder and Marker** (p.397) to assist in the determination of protein molecular weights. We recommend using unstained protein standards for precise determination of molecular weights in any buffer system. Prestained standards are recommended for monitoring the progress of the electrophoresis run and the efficiency of protein transfer to membranes during Western blot procedures. The quality of Fermentas unstained protein MW standards is confirmed by analysis on an Agilent 2100 bioanalyzer (*see* Fig.8.1).

Variation in Protein Mobility

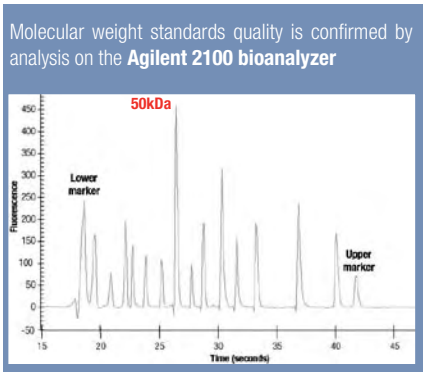
The apparent molecular weights of our prestained protein standards are calibrated in classical Tris-glycine-SDS Laemmli system. However, the prestained protein may have different mobility in other electrophoresis buffer and gel systems. Information on the migration pattern of a particular prestained protein standard on various gel types is provided on www.fermentas.com.

Modifications to natural proteins such as phosphorylation and glycosylation may alter protein mobility. The molecular weights of modified proteins do not always correspond to those of unmodified standard proteins of the same size.

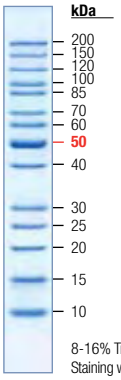
Protein Visualization (*see* p.406)

Proteins can be detected either within the polyacrylamide gel matrix or after their transfer to a polymeric membrane (p.407). Fermentas **PageBlue™ Protein Staining Solution** (#R0571) is based on the Coomassie Brilliant Blue G-250 dye and is recommended for fast, convenient gel and PVDF membrane staining. It detects as little as 5 ng of protein, and does not require a destaining step. For higher sensitivity staining and detection down to 0.05 ng of protein per band, Fermentas offers the new **PageSilver™ Silver Staining Kit** (#K0681).

A



B



8-16% Tris-glycine SDS-PAGE
Staining with PageBlue™ Protein Staining Solution (#R0571)

Figure 8.1. Analysis of PageRuler™ Unstained Protein Ladder (#SM0661):
A – on the Agilent 2100 bioanalyzer and Protein 200 Plus LabChip® Kit
B – on a 8-16% Tris-glycine SDS-PAGE

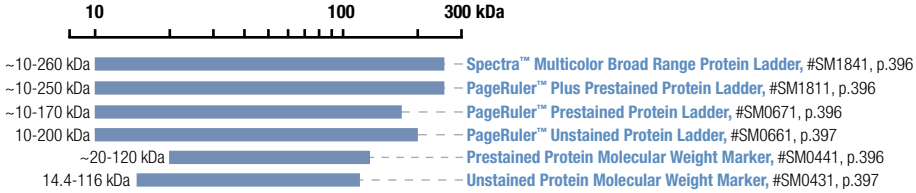
Protein Electrophoresis & Analysis: Selection Guide

Applications	Procedures	Products to use	Cat. #	Page
Protein extraction	Total protein extraction from mammalian cells or tissues	ProteoJET™ Mammalian Cell Lysis Reagent	K0301	392
	Nuclear and cytoplasmic protein extraction from mammalian cells or tissues	ProteoJET™ Cytoplasmic and Nuclear Protein Extraction Kit	K0311	393
Protein quantification	Colorimetric protein quantification	Bradford Reagent, ready-to-use	R1271	394
	Standard curve creation for protein quantification assays	Bovine Gamma Globulin Standard Set	R1291	395
		Bovine Serum Albumin Standard Set	R1281	395
		Protein Standard Solution	R0882	395
Protein electrophoresis	Monitoring protein separation; approximate sizing of proteins on SDS-PAGE	Spectra™ Multicolor Broad Range Protein Ladder	SM1841	396
		PageRuler™ Plus Prestained Protein Ladder	SM1811	396
		PageRuler™ Prestained Protein Ladder	SM0671	396
		Prestained Protein Molecular Weight Marker	SM0441	396
	Precise sizing of proteins on SDS-PAGE	PageRuler™ Unstained Protein Ladder	SM0661	397
		Unstained Protein Molecular Weight Marker	SM0431	397
	Preparation of protein samples for SDS-PAGE	DualColor™ Protein Loading Buffer Pack	R1011	400
		Protein Loading Buffer Pack	R0891	401
	SDS-PAGE	10X Tris-glycine-SDS Electrophoresis Buffer	B46	401
	SDS-PAGE of peptides and small proteins	10X Tris-tricine-SDS Electrophoresis Buffer	B48	401
	Native protein electrophoresis or Western transfer	10X Tris-glycine Electrophoresis Buffer	B47	401
Protein detection	Coomassie based protein staining in gels and PVDF membranes	PageBlue™ Protein Staining Solution	R0571	399
	Silver staining	PageSilver™ Silver Staining Kit	K0681	398
Western blotting	Monitoring protein transfer on membrane	Spectra™ Multicolor Broad Range Protein Ladder	SM1841	396
		PageRuler™ Plus Prestained Protein Ladder	SM1811	396
		PageRuler™ Prestained Protein Ladder	SM0671	396
		Prestained Protein Molecular Weight Marker	SM0441	396
		DualColor™ Protein Loading Buffer Pack	R1011	400
	Staining of proteins on PVDF membranes	PageBlue™ Protein Staining Solution	R0571	399

Protein Ladders and Markers: Selection Guide

Protein ladder/marker	Applications			Features				Detection		Cat. #	Page
	SDS-PAGE	Western blotting	Accurate MW estimation	MW range, kDa	Reference bands	Visibility without staining	Number of bands	PageBlue™ staining (#R0571)	PageSilver™ staining (#K0681)		
Spectra™ Multicolor Broad Range Protein Ladder	✓	✓	–	~10-260	✓	✓	10	not required	not required	SM1841	396
PageRuler™ Plus Prestained Protein Ladder	✓	✓	–	~10-250	✓	✓	9	not required	not required	SM1811	396
PageRuler™ Prestained Protein Ladder	✓	✓	–	~10-170	✓	✓	10	not required	not required	SM0671	396
PageRuler™ Unstained Protein Ladder	✓	✓	✓	10-200	✓	–	14	✓	✓	SM0661	397
Prestained Protein Molecular Weight Marker	✓	✓	–	~20-120	–	✓	6	not required	not required	SM0441	396
Unstained Protein Molecular Weight Marker	✓	✓	✓	14.4-116	–	–	7	✓	✓	SM0431	397

Range Selection Guide



Bulk quantities and custom formulations available upon request

Protein Extraction and Quantification

NEW

ProteoJET™ Mammalian Cell Lysis Reagent

#K0301 250 ml
(for 125 extractions from 100 µl of wet cell pellet or 500 extractions from 100 mg of tissue)

Related Products

- ProteoJET™ Cytoplasmic and Nuclear Protein Extraction Kit p.393
- Bradford Reagent, ready-to-use p.394
- Bovine Gamma Globulin Standard Set p.395
- Bovine Serum Albumin Standard Set p.395
- Protein Standard Solution p.395
- Prestained Protein Ladders and Markers p.396
- Unstained Protein Ladders and Markers p.397
- PageSilver™ Silver Staining Kit p.398
- PageBlue™ Protein Staining Solution p.399
- DualColor™ Protein Loading Buffer Pack p.400
- Protein Loading Buffer Pack p.401
- Protein Electrophoresis Buffers p.401
- DTT p.490

Description

The ProteoJET™ Mammalian Cell Lysis Reagent is a ready-to-use solution designed for extraction of native, non-denatured proteins from mammalian cultured cells or tissue samples. The procedure, which is based on lysis with mild detergents at room temperature, is fast and simple: no need for sonication, freeze thaw cycles or scraping of adherent cells.

The procedure yields proteins in their native, non-denatured functional state. Therefore, isolated proteins can be used directly in many proteomic and enzymatic activity applications, including immunoprecipitation, affinity purification, reporter gene assays and electrophoretic mobility shift assays (EMSA). Isolated proteins are also compatible with quantification assays such as Bradford and Lowry.

Features

- **High yield** total protein extraction from mammalian cultured cells or tissue samples.
- **Ready-to-use** formulation.
- **Convenient** – room temperature.
- **Easy-to-use** – no sonication, freeze-thaw cycles.
- **Compatible** with downstream applications:
  - protein quantification,
  - 1D and 2D electrophoresis,
  - immunoprecipitation and Western blotting,
  - EMSA, enzymatic activity and reporter gene assays.

Application

Extraction of total native, non-denatured protein from mammalian tissues or cultured cells (adherent and suspension).

Quality Control

Reagent is functionally tested for total protein extraction from cultured mammalian suspension cells.

Storage

Store at 4°C or at room temperature.

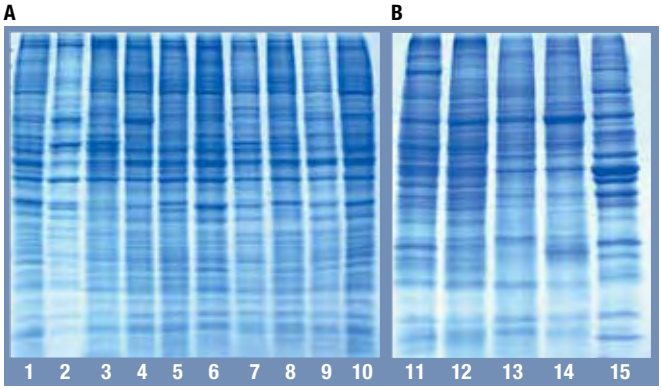


Figure 8.2. Total protein extraction.
Protein was extracted from various cell lines (**A**) and mouse tissues (**B**). Extracts were separated in 12% SDS-PAGE, gel stained with PageBlue™ Protein Staining Solution (#R0571).

A
1 – HEP2C cells
2 – human primary fibroblast cells
3 – NIH3T3 cells
4 – HEK293 cells
5 – Cos7 cells
6 – HeLa cells
7 – B50 cells
8 – CHO cells
9 – Raji cells
10 – Jurkat cells

B
11 – liver
12 – kidney
13 – spleen
14 – lung
15 – muscle

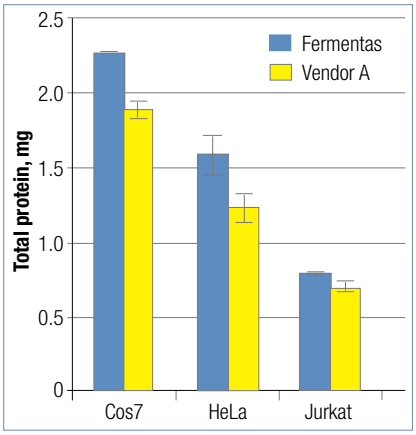


Figure 8.3. Comparison of total protein yields from 5x10⁶ cells.

Protocols and Recommendations

» Protein Samples: Extraction and Quantification Guides p.403

Ready-to-use | 4° Store at 4°C | RT Store at Room Temperature | -20° Store at -20°C

NEW

ProteoJET™ Cytoplasmic and Nuclear Protein Extraction Kit 4°

#K0311 for 50 preps

Related Products

- ProteoJET™ Mammalian Cell Lysis Reagent p.392
- Bradford Reagent, ready-to-use p.394
- Bovine Gamma Globulin Standard Set p.395
- Bovine Serum Albumin Standard Set p.395
- Protein Standard Solution p.395
- Prestained Protein Ladders and Markers p.396
- Unstained Protein Ladders and Markers p.397
- PageSilver™ Silver Staining Kit p.398
- PageBlue™ Protein Staining Solution p.399
- DualColor™ Protein Loading Buffer Pack p.400
- Protein Loading Buffer Pack p.401
- Protein Electrophoresis Buffers p.401
- DTT p.490

Description

The ProteoJET™ Cytoplasmic and Nuclear Protein Extraction Kit is designed for rapid stepwise isolation of native, non-denatured cytoplasmic proteins and intact nuclei from cultured cells or tissue samples. The procedure, which is based on lysis with mild detergents, is fast and simple. The isolated proteins are ready for direct use in many proteomic or enzymatic activity applications, including immunoprecipitation, affinity purification and reporter gene assays. Cell extracts prepared using this kit are compatible with common downstream applications, including 1D and 2D electrophoresis, Western blotting and electrophoretic mobility shift assays (EMSA). Isolated proteins are also compatible with quantification assays such as Bradford and Lowry.

The extracted nuclei can either be stored frozen in the provided Nuclei Storage Buffer or lysed with Nuclei Lysis Reagent. The intact nuclei further may be used for isolation of chromatin, histones and nuclear RNA. Nuclear extracts are used in DNA-protein interaction assays (EMSA), 2D fractionation or other applications.

The kit contains sufficient reagents for extraction of cytoplasmic and nuclear fractions from 50 samples of 50 µl wet cell pellet or 100 mg of tissue.

Features

- **Versatile** – isolates intact nuclei and cytoplasmic protein fraction from the same cell or tissue sample.
- **High quality fractionation** – no cross-contamination between cytoplasmic and nuclear fractions.
- **Fast** – procedure takes less than one hour.
- **Compatible** with downstream applications:
  - protein quantification,
  - 1D and 2D electrophoresis,
  - immunoprecipitation and Western blotting,
  - EMSA, enzymatic activity and reporter gene assays.

Application

Preparation of native, non-denatured nuclear and cytoplasmic protein extracts from mammalian cultured cells or tissue samples.

Quality Control

The kit is functionally tested for cytoplasmic and nuclear protein extraction from cultured mammalian suspension cells.

Components of the Kit

- Cell Lysis Buffer
- Nuclei Washing Buffer
- Nuclei Storage Buffer
- Nuclei Lysis Reagent
- 1 M DTT
- Detailed Protocol

Storage

All components of the kit, except 1 M DTT, can be stored at 4°C for at least one year. Store 1 M DTT in aliquots at -20°C.

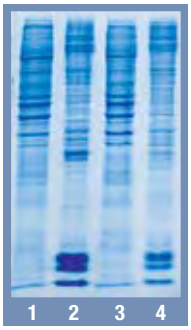


Figure 8.4. Cytoplasmic and nuclear proteins extracted using the ProteoJET™ Cytoplasmic and Nuclear Protein Extraction Kit from various cell lines.
10 µg of cytoplasmic proteins and 5 µl of purified nuclei were separated in 12% SDS-PAGE, gel stained with PageBlue™ Protein Staining Solution (#R0571).
1 – cytoplasmic proteins from Jurkat cells
2 – nuclear proteins from Jurkat cells
3 – cytoplasmic proteins from HeLa cells
4 – nuclear proteins from HeLa cells

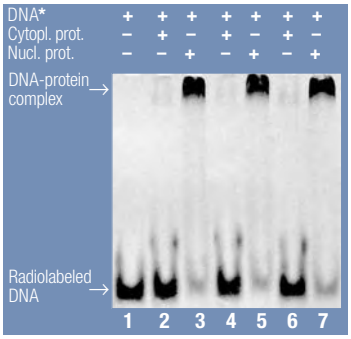


Figure 8.5. EMSA of cytoplasmic and nuclear proteins extracted from various cell lines.
* [γ^{33}-P] labeled DNA containing AP-1 transcription factor binding sequence.
1 – no protein added
2 – cytoplasmic proteins from Cos7 cells
3 – nuclear proteins from Cos7 cells
4 – cytoplasmic proteins from NIH3T3 cells
5 – nuclear proteins from NIH3T3 cells
6 – cytoplasmic proteins from HeLa cells
7 – nuclear proteins from HeLa cells

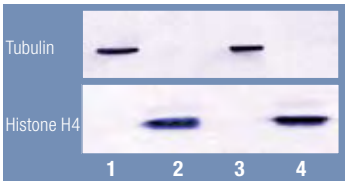


Figure 8.6. Western blot analysis demonstrating pure cytoplasmic and nuclear protein fractions.
5 µg of cytoplasmic protein and 10 µl of purified nuclei were separated in 12% SDS-PAGE. The cytoplasmic protein tubulin and nuclear protein histone H4 were detected using specific antibodies.
1 – cytoplasmic proteins from Jurkat cells
2 – nuclear proteins from Jurkat cells
3 – cytoplasmic proteins from HeLa cells
4 – nuclear proteins from HeLa cells

Protocols and Recommendations

» Protein Samples: Extraction and Quantification Guides p.403

NEW

Bradford Reagent, ready-to-use

4°

#R1271 1 liter
(for 1000 standard test tube assays
or 4000 standard microplate assays)

Related Products

- ProteoJET™ Mammalian Cell Lysis Reagent p.392
- ProteoJET™ Cytoplasmic and Nuclear Protein Extraction Kit p.393
- Bovine Gamma Globulin Standard Set, ready-to-use p.395
- Bovine Serum Albumin Standard Set, ready-to-use p.395
- Protein Standard Solution p.395
- Prestained Protein Ladders and Markers p.396
- Unstained Protein Ladders and Markers p.397
- PageSilver™ Silver Staining Kit p.398
- PageBlue™ Protein Staining Solution p.399
- DualColor™ Protein Loading Buffer Pack p.400
- Protein Loading Buffer Pack p.401
- Protein Electrophoresis Buffers p.401
- DTT p.490

Description

Bradford Reagent, ready-to-use, is a convenient 1X concentrated dye reagent for rapid and accurate estimation of total protein concentration in solution.

The Bradford protein assay relies on the formation of complexes between Coomassie Brilliant Blue G-250 dye and proteins in solution. The binding of the dye to the protein causes a shift in the absorption maximum of the dye from 465 nm to 595 nm (1, 2). Unknown protein concentrations are determined by reference of their absorptions to absorptions obtained for series of standard protein dilutions assayed in parallel with the unknown samples.

The quantification procedure is simple and rapid. The dye binding process is complete in approximately 5 min with color stability for 1 hour.

The absorption is proportional to the amount of protein present in the solution. The working range is 1-2000 µg/ml of protein.

Depending on the protein concentration, standard or microassay procedures can be used. Depending on the volume of protein sample available, the assay can be performed in test tubes or microplates.

Features

- **Ready-to-use formulation** – no dilution is required.
- **Rapid color development**.
- **Broad working concentration range** – 1-2000 µg/ml.
- **Compatible** with a variety of substances present in samples, including solvents, salts, detergents, thiols, reducing and chelating agents.

Application

Rapid and accurate estimation of protein concentration in solution.

Quality Control

Functionally tested for accurate determination of various protein concentrations.

Storage

Store protected from light at 4°C for one year.

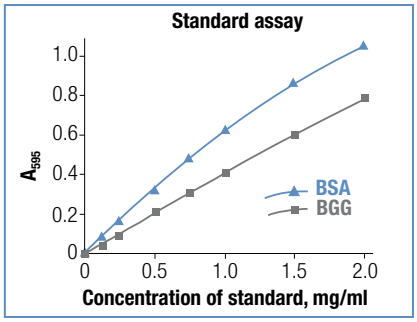


Figure 8.7. Standard curve obtained using Bradford reagent, ready-to-use, in standard assay.
BSA – bovine serum albumin.
BGG – bovine gamma globulin.

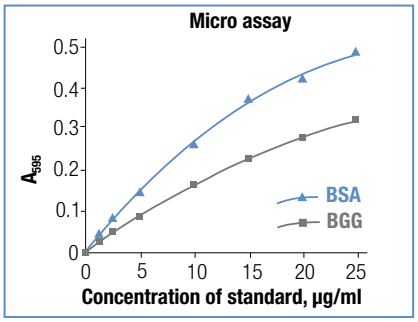


Figure 8.8. Standard curve obtained using Bradford reagent, ready-to-use, in micro assay.
BSA – bovine serum albumin.
BGG – bovine gamma globulin.

Protocols and Recommendations

» Protein Samples: Extraction and Quantification Guides p.403
» Troubleshooting Guide p.408

References

1. Bradford, M.M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem., 72, 248-54, 1976.
2. Sedmak, J.J., Grossberg, S.E., A rapid, sensitive and versatile assay for protein using Coomassie brilliant blue G-250, Anal. Biochem., 79, 544-52, 1977.

NEW

Protein Standard Sets, ready-to-use

4°

Bovine Serum Albumin Standard Set, ready-to-use

#R1281

Bovine Gamma Globulin Standard Set, ready-to-use

#R1291

7 concentrations in the range of 0.125-2.0 mg/ml, 4x1 ml each, for 200 standard test tube assays or 800 standard microplate assays

Related Products

- ProteoJET™ Mammalian Cell Lysis Reagent p.392
- ProteoJET™ Cytoplasmic and Nuclear Protein Extraction Kit p.393
- Bradford Reagent, ready-to-use p.394
- Protein Standard Solution p.395
- Prestained Protein Ladders and Markers p.396
- Unstained Protein Ladders and Markers p.397
- PageSilver™ Silver Staining Kit p.398
- PageBlue™ Protein Staining Solution p.399
- DualColor™ Protein Loading Buffer Pack p.400
- Protein Loading Buffer Pack p.401
- Protein Electrophoresis Buffers p.401
- DTT p.490

Description

The Bovine Serum Albumin (BSA) and Bovine Gamma Globulin (BGG) Standard Sets contain seven pre-diluted protein concentrations (0.125, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0 mg/ml) ideal for reliable and accurate total protein quantification. Each set contains four aliquots of every BSA or BGG concentration, grouped by the color of the screw cap vial. This permits easy identification of each protein concentration and eliminates time-consuming standard dilution set up procedures. BSA and BGG pre-diluted standards, when used together with our ready-to-use Bradford Reagent (#R1271), allow for single step determination of total protein concentration.
Each set contains 7 concentrations in the range of 0.125-2.0 mg/ml, 4x1 ml each, for 200 standard tube assays or 800 standard microplate assays.

References

1. Bradford, M.M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem., 72, 248-54, 1976.
2. Brown, J.R., Structure of bovine serum albumin, Fed. Proc., 34, 591, 1975.

Features

- **Reliable and accurate** determination of protein concentration.
- **Convenient** – ready-to-use concentrations packed in different colored screw cap vials.
- **Provided in aliquots** – ensures protein stability and minimizes the possibility of contamination.

Application

Standard curve creation for protein quantification assays (e.g. Bradford, *see* Fig.8.7 on p.394 (1)).

Quality Control

Functionally tested in Bradford assay using Bradford Reagent, ready-to-use (#R1271).

Molecular Weight

Bovine Serum Albumin – 66.2 kDa (2);
Bovine Gamma Globulin – 140 kDa.

Composition

Different concentrations of BSA or BGG in 0.15 M NaCl, containing 0.05% NaN_3 as preservative.

Storage

Store at 4°C.

Protein Standard Solution

-20°

#R0882 10x1 ml

Related Products

- ProteoJET™ Mammalian Cell Lysis Reagent p.392
- ProteoJET™ Cytoplasmic and Nuclear Protein Extraction Kit p.393
- Bradford Reagent, ready-to-use p.394
- Bovine Gamma Globulin Standard Set p.395
- Bovine Serum Albumin Standard Set p.395
- Prestained Protein Ladders and Markers p.396
- Unstained Protein Ladders and Markers p.397
- PageSilver™ Silver Staining Kit p.398
- PageBlue™ Protein Staining Solution p.399
- DualColor™ Protein Loading Buffer Pack p.400
- Protein Loading Buffer Pack p.401
- Protein Electrophoresis Buffers p.401
- DTT p.490

Description

Our Bovine Serum Albumin (BSA)-based Protein Standard Solution is ideal to use as a standard for colorimetric determination of total protein concentration by Bradford (1), Lowry (2) or other protein quantification assays.

References

1. Bradford, M.M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem., 72, 248-54, 1976.
2. Lowry, O.H., et al., Protein measurement with the Folin phenol reagent, J. Biol. Chem., 193, 265, 1951.
3. Brown, J.R., Structure of bovine serum albumin, Fed. Proc., 34, 591, 1975.

Application

Standard for protein quantification.

Quality Control

Total protein is determined by the Lowry method (2).

Molecular Weight

66.2 kDa (3).

Composition

200 µg/ml BSA in 0.15 M NaCl, containing 0.05% NaN_3 as a preservative.

Storage

Store at -20°C.

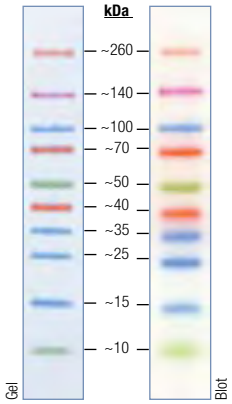
Protocols and Recommendations

» Protein Samples: Extraction and Quantification Guides p.403

Prestained Protein Ladders and Markers

NEW Spectra™ Multicolor Broad Range Protein Ladder

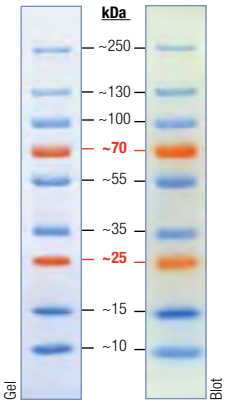
#SM1841 2x250 µl
(for 50 mini gel applications, 10 µl/well
or 25 large gel applications, 20 µl/well)



4-20% Tris-glycine SDS-PAGE

PageRuler™ Plus Prestained Protein Ladder

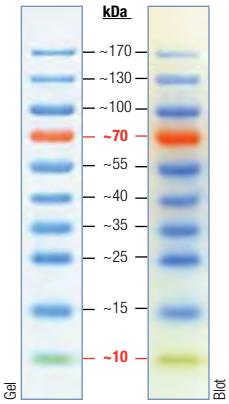
#SM1811 2x250 µl
(for 100 mini gel applications, 5 µl/well
or 50 large gel applications, 10 µl/well)



4-20% Tris-glycine SDS-PAGE

PageRuler™ Prestained Protein Ladder

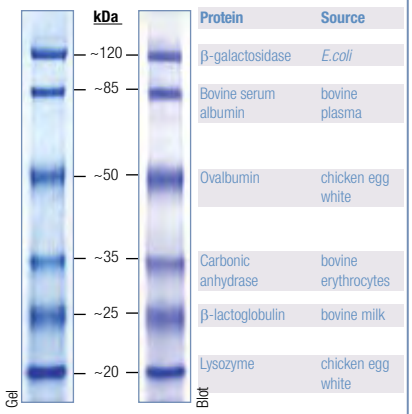
#SM0671 2x250 µl
(for 100 mini gel applications, 5 µl/well
or 50 large gel applications, 10 µl/well)



4-20% Tris-glycine SDS-PAGE

Prestained Protein Molecular Weight Marker

#SM0441 2x250 µl
(for 100 mini gel applications, 5 µl/well
or 50 large gel applications, 10 µl/well)



8-16% Tris-glycine SDS-PAGE

Related Products

- ProteoJET™ Mammalian Cell Lysis Reagent p.392
- ProteoJET™ Cytoplasmic and Nuclear Protein Extraction Kit p.393
- Bradford Reagent, ready-to-use p.394
- Protein Standard Sets p.395
- Protein Standard Solution p.395
- Unstained Protein Ladders and Markers p.397
- PageSilver™ Silver Staining Kit p.398
- PageBlue™ Protein Staining Solution p.399
- DualColor™ Protein Loading Buffer Pack p.400
- Protein Loading Buffer Pack p.401
- Protein Electrophoresis Buffers p.401
- DTT p.490

Description

Prestained protein ladders/marker are designed for monitoring protein separation during SDS-polyacrylamide gel electrophoresis, verification of Western transfer efficiency on PVDF, nylon and nitrocellulose membranes and for approximate sizing of proteins (1-3). The prestained protein ladders are composed of prestained recombinant prokaryotic proteins. The prestained protein marker is a mixture of prestained natural proteins.

Spectra™ Multicolor Broad Range Protein Ladder is a 4-color protein standard with 10 proteins covering a wide range molecular weights from 10 to 260 kDa. Four different chromophores are bound to the proteins producing a brightly colored ladder with an easy-to-remember pattern.

PageRuler™ Plus Prestained Protein Ladder is a dual color ladder with 9 proteins covering a wide range of molecular weights from 10 to 250 kDa. The ladder contains two orange reference bands at 70 kDa and 25 kDa.

PageRuler™ Prestained Protein Ladder is a 3-color ladder with 10 proteins covering a molecular weight range from 10 to 170 kDa. The ladder contains one orange reference band at 70 kDa and one green band at 10 kDa.

Prestained Protein Molecular Weight Marker is a mixture of 6 prestained natural proteins with apparent molecular weights from 20 kDa to 120 kDa.

Features

- **Sharp bands**.
- **Stable colors**.
- **Bright reference bands**.
- **Broad range**.
- **Ready-to-use** – supplied in a loading buffer for direct loading on gels.

Applications

- Monitoring protein migration during SDS-polyacrylamide gel electrophoresis.
- Monitoring protein transfer onto membranes after Western blotting.
- Sizing of proteins on SDS-polyacrylamide gels and Western blots.

Composition

Ladders: 62.5 mM Tris-H_3PO_4 (pH 7.5 at 25°C), 1 mM EDTA, 2% SDS, 10 mM DTT, 1 mM NaN_3 and 33% glycerol.
Marker: 62.5 mM Tris-HCl (pH 7.5 at 25°C), 1 mM EDTA, 2% SDS, 10 mM DTT, 1.5 mM NaN_3 and 33% glycerol.

Quality Control

Tested in SDS-polyacrylamide gel electrophoresis and Western blotting.

Storage

Ladders: stable at 4°C for up to 3 months. For long term storage, store at -20°C.
Marker: store at -20°C.

References

1. Laemmli, U.K., Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature, 227, 680-685, 1970.
2. Burnette, W.N., "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate – polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A, Anal. Biochem., 112 (2), 195-203, 1981.
3. Towbin, H., et al., Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications, Proc. Natl. Acad. Sci. USA, 76, 4350-4354, 1979.

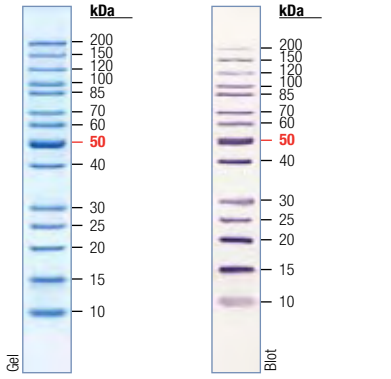
Ready-to-use | 4° Store at 4°C | RT Store at Room Temperature | -20° Store at -20°C

Unstained Protein Ladders and Markers

-20°

PageRuler™ Unstained Protein Ladder

#SM0661 2x250 µl
(for 100 mini gel applications, 5 µl/well
or 50 large gel applications, 10 µl/well)

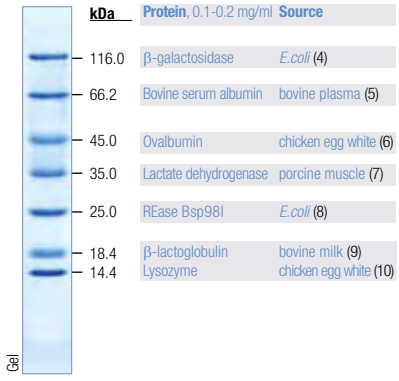


8-16% Tris-glycine SDS-PAGE Staining with PageBlue™ Protein Staining Solution (#R0571)

8-16% Tris-glycine SDS-PAGE Detection with *Strep*-Tactin®-AP conjugate

Unstained Protein Molecular Weight Marker

#SM0431 2x1000 µl
(for 400 mini gel applications, 5 µl/well
or 200 large gel applications, 10 µl/well)



8-16% Tris-glycine SDS-PAGE
Staining with PageBlue™ Protein Staining Solution (#R0571)

Related Products

- ProteoJET™ Mammalian Cell Lysis Reagent p.392
- ProteoJET™ Cytoplasmic and Nuclear Protein Extraction Kit p.393
- Bradford Reagent, ready-to-use p.394
- Protein Standard Sets p.395
- Protein Standard Solution p.395
- Prestained Protein Ladders and Markers p.397
- PageSilver™ Silver Staining Kit p.398
- PageBlue™ Protein Staining Solution p.399
- DualColor™ Protein Loading Buffer Pack p.400
- Protein Loading Buffer Pack p.401
- Protein Electrophoresis Buffers p.401
- DTT p.490

Description

Unstained protein ladders and markers are designed for accurate sizing of proteins in SDS-polyacrylamide gel electrophoresis (1), as well as on PVDF, nylon and nitrocellulose membranes.

PageRuler™ Unstained Protein Ladder is a mixture of 14 recombinant, highly purified, unstained proteins ranging in size from 10 kDa to 200 kDa. Each protein in the ladder contains an integral Strep-tag® II sequence which can be detected directly on Western blots using a Strep-Tactin®-AP* conjugate or an antibody against Strep-tag® II (2, 3). Strep-tag® II detection systems are not supplied by Fermentas.

Unstained Protein Molecular Weight Marker is a mixture of 7 native proteins (14.4-116 kDa). Our unstained protein ladder and marker produce sharp bands on SDS-polyacrylamide gel following staining of the gel with PageBlue™ Protein Staining Solution (#R0571), PageSilver™ Silver Staining Kit (#K0681) or by other protein staining methods. PageBlue™ Protein Staining Solution can also be used to visualize unstained ladders and markers on PVDF membranes.

Features

- **Broad range**.
- **Ready-to-use** – supplied in a loading buffer for direct loading on gels.
- **Sharp bands**, reference band included in the PageRuler™ ladder.
- Each protein in the PageRuler™ ladder contains an integral Strep-tag® II sequence.

Application

Accurate protein sizing on SDS-polyacrylamide gels and Western blots.

Composition

Ladder: 0.02-0.05 mg/ml of each protein in 62.5 mM Tris-H_3PO_4 (pH 7.5 at 25°C), 1 mM EDTA, 2% SDS, 100 mM DTT, 1 mM NaN_3, 0.01% bromophenol blue and 33% glycerol.
Marker: 0.1-0.2 mg/ml of each protein in 62.5 mM Tris-HCl (pH 7.0 at 25°C), 1 mM EDTA, 2% SDS, 50 mM DTT, 30 mM NaCl, 1 mM NaN_3, 0.01% bromophenol blue and 50% glycerol.

Quality Control

Tested in SDS-polyacrylamide gel electrophoresis and Western blotting.

Storage

Store at -20°C.

References

1. Laemmli, U.K., Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature, 227, 680-685, 1970.
2. Schmidt, T.G.M., Skerra, A., The random peptide library-assisted engineering of a C-terminal affinity peptide, useful for the detection and purification of a functional Ig Fv fragment, Prot. Engineering, 6, 109-122, 1993.
3. Tsiotis, G., et al., Isolation and structural characterization of trimeric cyanobacterial photosystem I complex with the help of recombinant antibody fragments, Eur. J. Biochem., 231, 823-830, 1995.
4. Fowler, A.V., Zabin, I., The amino acid sequence of beta-galactosidase of *Escherichia coli*, Proc. Natl. Acad. Sci. USA, 74, 1507-1510, 1977.
5. Brown, J.R., Structure of bovine serum albumin, Fed. Proc., 34, 591, 1975.
6. Warner, R.C., Egg proteins, Proteins, II A., 435, (Neurath, H., Bailey, K., eds.), Academic Press, N.Y., 1954.
7. Castellino, F.J., Barker, R., Examination of the dissociation of multichain proteins in guanidine hydrochloride by membrane osmometry, Biochemistry, 7, 2207-2217, 1968.
8. Unpublished results.
9. Dayhoff, M., Atlas of Protein Sequence and Structure, vol.4, National Biomedical Research Foundation, Silver Spring, M.D.,1969.
10. Jolles, P., Lysozymes: A chapter of molecular biology, Angew. Chem., Intl. Edit., 8, 227-294, 1969.

Protocols and Recommendations

- » Recommendations for Loading p.403
- » SDS-PAGE Protocol p.404
- » Semi-dry Protein Transfer for Western Blotting p.407
- » Gel Staining p.405
- » Troubleshooting Guide p.408

* *Strep*-tag® technology for protein purification and detection is covered by US patent 5,506,121, UK patent 2272698 and French patent 93 13 066. *Strep*-Tactin® is covered by US patent 6,103,493.

Protein Detection

NEW

PageSilver™ Silver Staining Kit

RT

#K0681 for 25 mini gels

Related Products

- ProteoJET™ Mammalian Cell Lysis Reagent p.392
- ProteoJET™ Cytoplasmic and Nuclear Protein Extraction Kit p.393
- Bradford Reagent, ready-to-use p.394
- Bovine Gamma Globulin Standard Set p.395
- Bovine Serum Albumin Standard Set p.395
- Protein Standard Solution p.395
- Prestained Protein Ladders and Markers p.396
- Unstained Protein Ladders and Markers p.397
- PageBlue™ Protein Staining Solution p.399
- DualColor™ Protein Loading Buffer Pack p.400
- Protein Loading Buffer Pack p.401
- Protein Electrophoresis Buffers p.401
- DTT p.490

Description

The PageSilver™ Silver Staining Kit is a complete system for extremely sensitive and rapid staining of protein, DNA, RNA in polyacrylamide gels. The procedure is based on the use of silver nitrate to bind proteins at a weakly acidic pH and subsequent reduction of silver ions to metallic silver by formaldehyde at an alkaline pH (1, 2, 3). The staining procedure is fast, simple and can be completed in approximately 60 minutes with very little hands-on time. All components of the PageSilver™ Silver Staining Kit are optimized for maximum sensitivity of protein staining (Fig.8.10) with a clear background (Fig.8.9). As little as 0.05-0.6 ng of protein per band can be visualized. This reflects a >100 times higher sensitivity compared to traditional Coomassie Brilliant Blue R250 staining and allows for superior detection of low abundance proteins.

Features

- **High sensitivity** – 0.05 ng of protein per band can be detected (Fig.8.10) when the conventional protocol is used (p.406).
- **Clear background** (Fig.8.9).
- **Fast and easy staining** – procedure takes only 60 minutes when the fast protocol is used (p.406).
- **Compatible** with *in situ* zymography.

Applications

- Highly sensitive staining of proteins, DNA and RNA in polyacrylamide gels.
- Stains both non-denaturing or denaturing polyacrylamide gels containing SDS or urea.
- Visualization of nanogram quantities of DNA and RNA.

Quality Control

The kit is tested using the staining protocol for maximum speed, different dilutions of BSA (0.1-2.0 ng) are electrophoresed on a 1.0 mm. 12% Tris-glycine SDS gel. Silver staining detects 0.1 ng BSA in clear background.

Components of the Kit

- Sensitizer concentrate
- Staining reagent
- Developing reagent
- Formaldehyde
- Stop reagent
- Detailed Protocol

Note

Analysis of stained proteins by mass spectrometry is not recommended.

References

1. Rabilloud, T. et al., Silver-staining of proteins in polyacrylamide gels: a general overview, Cell. Mol. Biol., 40, 57-75, 1994. Cell. Mol. Biol. 40, 57-75, 1994.
2. Blum, H., Beier, H. And Gross, H.J., Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels, Electrophoresis, 8, 93-99, 1987.
3. Sorense, B.K., et al., Silver Staining of Proteins on Electroblotting Membranes and Intensification of Silver Staining of Proteins Separated by Polyacrylamide Gel Electrophoresis, Anal.Biochem., 304, 33-41, 2002.

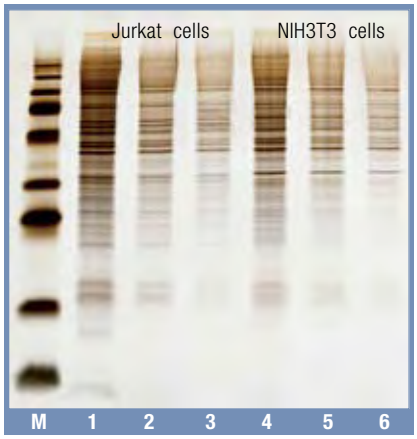


Figure 8.9. Highly sensitive protein staining in a clear background with PageSilver™ Silver Staining Kit.
Decreasing amounts of cell lysates were separated on 4-12% Tris-glycine SDS-PAGE and stained with the PageSilver™ Silver Staining Kit.
M – PageRuler™ Plus Prestained Protein Ladder (#SM1811)
1, 4 – 500 ng of total protein
2, 5 – 200 ng of total protein
3, 6 – 100 ng of total protein

Protocols and Recommendations

- Protein Detection: Selection Guide p.403
- Staining Protocol p.406
- Protein Ladders/Markers: Recommendations for Loading p.403
- Protein Samples: Extraction and Quantification Guides p.403
- Semi-dry Protein Transfer for Western Blotting p.407
- Troubleshooting Guide p.408

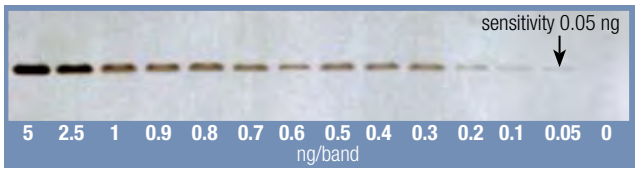


Figure 8.10. High sensitivity of staining with PageSilver™ Silver Staining Kit.
Different amounts of BSA were run on 4-12% Tris-glycine SDS-PAGE and stained with the PageSilver™ Silver Staining Kit according to protocol for maximum sensitivity (*see* p.406).

Ready-to-use | 4° Store at 4°C | RT Store at Room Temperature | -20° Store at -20°C

PageBlue™ Protein Staining Solution

RT

#R0571 1 liter
(for up to 150 mini gels)

Related Products

- ProteoJET™ Mammalian Cell Lysis Reagent p.392
- ProteoJET™ Cytoplasmic and Nuclear Protein Extraction Kit p.393
- Bradford Reagent, ready-to-use p.394
- Bovine Gamma Globulin Standard Set p.395
- Bovine Serum Albumin Standard Set p.395
- Protein Standard Solution p.395
- Prestained Protein Ladders and Markers p.396
- Unstained Protein Ladders and Markers p.397
- PageSilver™ Silver Staining Kit p.398
- DualColor™ Protein Loading Buffer Pack p.400
- Protein Loading Buffer Pack p.401
- Protein Electrophoresis Buffers p.401
- DTT p.490

Description

PageBlue™ Protein Staining Solution is a ready-to-use solution for fast and sensitive staining of proteins separated in polyacrylamide gels or PVDF membranes. PageBlue™ is based on the Coomassie Brilliant Blue G-250 dye.

PageBlue™ dye forms colloidal particles allowing proteins to be preferentially stained eliminating the need for laborious, expensive and hazardous gel de-staining procedures. Proteins are stained to an endpoint, therefore over-staining does not occur even after overnight staining.

The linear dynamic range of PageBlue™ extends over two orders of magnitude (5-500 ng, *see* picture below) and is about 10 times more sensitive than traditional Coomassie Brilliant Blue R250.

PageBlue™ staining solution does not contain methanol or acetic acid and can be reused up to three times without any loss in sensitivity.

Features

- **Ready-to-use**.
- **Easy-to-use** – simply soak the gel in the solution and observe stained bands. No destaining required.
- **Safe** – does not contain methanol or acetic acid.
- **End point stain** – cannot overstain even after overnight incubation.
- **Highly sensitive** – as little as 5 ng of protein can be detected. More sensitive than Coomassie Brilliant Blue R-250.
- **Broad linear dynamic range**.
- **Fast** – 25-40 minute protocol.
- **Compatible** with:
  - mass spectrometry;
  - silver staining;
  - PVDF membranes.
- **Cost efficient** – can be reused up to 3 times.

Applications

- Visualization and quantification of proteins separated in 1D, 2D, IEF polyacrylamide gels.
- Protein staining after transfer onto PVDF membranes.
- *In situ* zymography.

Quality Control

Tested in staining of the Protein Molecular Weight Marker (#SM0431) on SDS polyacrylamide gel.

Storage

Stable at 4 to 26°C.

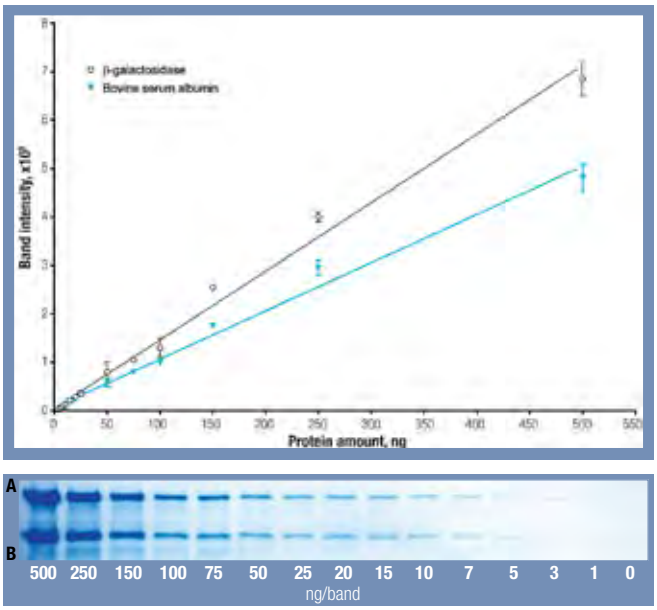


Figure 8.11. Sensitivity and linear dynamic range of PageBlue™ Protein Staining Solution.
A – β-galactosidase
B – bovine serum albumin
Electrophoresis conditions: 12% Tris-glycine SDS-PAGE, 0.75 mm gel, well width 3 mm.

Protocols and Recommendations

- Protein Ladders/Markers: Recommendations for Loading p.403
- Staining Protocol p.405
- Semi-dry Protein Transfer for Western Blotting p.407
- Membrane Staining and Destaining p.407
- Troubleshooting Guide p.408

Bulk quantities and custom formulations available upon request

Buffers for Protein Sample Loading

NEW

DualColor™ Protein Loading Buffer Pack -20°

#R1011 for 1000 samples of 40 µl
4X DualColor™ Protein Loading Buffer 2x5 ml
20X Reducing Agent (2 M DTT) 2x1.0 ml

Related Products

- ProteoJET™ Mammalian Cell Lysis Reagent p.392
- ProteoJET™ Cytoplasmic and Nuclear Protein Extraction Kit p.393
- Bradford Reagent, ready-to-use p.394
- Bovine Gamma Globulin Standard Set p.395
- Bovine Serum Albumin Standard Set p.395
- Protein Standard Solution p.395
- Prestained Protein Ladders and Markers p.396
- Unstained Protein Ladders and Markers p.397
- PageSilver™ Silver Staining Kit p.398
- PageBlue™ Protein Staining Solution p.399
- Protein Loading Buffer Pack p.401
- Protein Electrophoresis Buffers p.401
- DTT p.490

Reference

1. Electrophoresis in practice, 4th edition, Westermeier, R., Wiley-VCH, 2005.

Description

The DualColor™ Protein Loading Buffer Pack is a complete solution for the preparation of protein samples prior to SDS-PAGE. Many proteins are sensitive to pH changes that result from temperature fluetuations during electrophoresis in Tris buffers. This optimized loading buffer prevents protein degradation during sample heating prior to SDS-PAGE (1) as well as during the electrophoresis run.

The loading buffer contains two tracking dyes; blue (bromophenol blue) and pink (pyronin Y) which allow for tracking the progress of electrophoresis. In addition, the pink dye permits monitoring of protein transfer to the membrane during Western blot procedures.

The DualColor™ Protein Loading Buffer Pack also contains SDS and DTT for complete disruption of all high-order protein structures. The SDS included in the buffer binds to hydrophobic regions of the protein, causing the protein to unfold and giving it a negative charge. DTT breaks disulfide bonds and destroys residual secondary structures. As DTT is prone to oxidation during multiple freeze-thaw cycles it is supplied in separate vials. Glycerol is included in the loading buffer to ensure protein samples remain in the gel wells after loading.

Features

- **Protects** proteins from degradation during sample preparation for SDS-PAGE.
- **Convenient** two dyes (blue and pink) allow for:
  - tracking the progress of electrophoresis,
  - monitoring Western blot protein transfer.

Applications

- Preparation of proteins for SDS-polyacrylamide gel electrophoresis (SDS-PAGE).
- Monitoring protein transfer during Western blotting.

Composition

- **4X Loading Buffer:** 0.25 M Tris-HCl (pH 8.5 at 25°C), 8% SDS, 1.6 mM EDTA, 0.024% Pyronin Y, 0.04% Bromophenol blue and 40% glycerol.
- **20X Reducing Agent:** 2 M DTT.

Quality Control

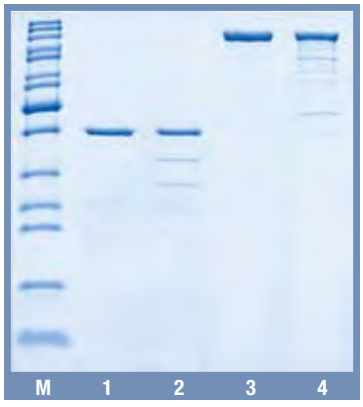
Tested in protein samples preparation prior to SDS-PAGE and subsequent Western blotting.

Note

In 15% PAA gels pyronin Y dye migrates slower than bromophenol blue.

Storage

Store 4X DualColor™ Protein Loading Buffer at room temperature or at -20°C for long term storage.
Store 20X Reducing Agent at -20°C.



M – PageRuler™ Unstained Protein Ladder (#SM0661)
1, 3 – protein sample prepared with DualColor™ Protein Loading buffer (pH 8.5) and DTT
2, 4 – protein sample prepared with conventional Laemmli loading buffer (pH 6.8) and DTT

Figure 8.12. Increased protein stability with DulaColor™ Protein Loading Buffer.
12% Tris-glycine SDS-PAGE, proteins stained with PageBlue™ Protein Staining Solution (#R0571).

Protocols and Recommendations

» Protein Samples: Extraction and Quantification Guides p.403
» Protein Samples: Preparation for Loading on SDS-PAGE p.404
» Semi-dry Protein Transfer for Western Blotting p.407
» Troubleshooting Guide p.408

1-2 – protein samples prepared with DualColor™ Protein Loading Buffer Pack
3-4 – protein samples prepared with Protein Loading Buffer Pack (#R0891)

Figure 8.13. Visibility of the DualColor™ Protein Loading Buffer dyes.
12% Tris-glycine SDS-PAGE.

Ready-to-use | 4° Store at 4°C | RT Store at Room Temperature | -20° Store at -20°C

Protein Loading Buffer Pack

-20°

#R0891 for 2000 samples of 50 µl

5X Protein Loading Buffer	20 ml
20X Reducing Agent (2 M DTT)	4x1.5 ml

Related Products

- ProteoJET™ Mammalian Cell Lysis Reagent p.392
- ProteoJET™ Cytoplasmic and Nuclear Protein Extraction Kit p.393
- Bradford Reagent, ready-to-use p.394
- Bovine Gamma Globulin Standard Set p.395
- Bovine Serum Albumin Standard Set p.395
- Protein Standard Solution p.395
- Prestained Protein Ladders and Markers p.396
- Unstained Protein Ladders and Markers p.397
- PageSilver™ Silver Staining Kit p.398
- PageBlue™ Protein Staining Solution p.399
- DualColor™ Protein Loading Buffer Pack p.400
- Protein Electrophoresis Buffers p.401
- DTT p.490

Description

The Protein Loading Buffer Pack is used for preparation of protein samples for denaturing SDS-polyacrylamide gel electrophoresis. This buffer contains all the necessary reagents for complete disruption of high-order protein structures. The SDS included in the buffer binds to hydrophobic regions of the protein, causing the protein to unfold and giving it a negative charge. DTT breaks disulfide bonds and destroys residual secondary structures. As DTT is prone to oxidation during multiple freeze-thaw cycles it is supplied in separate vials. Glycerol is included in the loading buffer to ensure protein samples remain in gel wells after loading. The bromophenol blue dye allows for visualization of probes and monitoring the progress of electrophoresis.

Application

Preparation proteins for SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

Composition

- **5X Loading Buffer:** 0.313 M Tris-HCl (pH 6.8 at 25°C), 10% SDS, 0.05% bromophenol blue and 50% glycerol.
- **20X Reducing Agent:** 2 M DTT.

Quality Control

Tested in protein sample preparation prior to SDS-PAGE.

Storage

5X Protein Loading Buffer store at -20°C or at 4°C.
20X Reducing Agent store at -20°C.

Protocols and Recommendations

» Protein Ladders/Markers: Recommendations for Loading p.403
» Protein Samples: Extraction and Quantification Guides p.403
» Protein Samples: Preparation for Loading on SDS-PAGE p.404
» Troubleshooting Guide p.408

Buffers for Protein Electrophoresis

Protein Electrophoresis Buffers

RT

Buffer	Cat. #	Size	1X composition	Applications	Usage recommendations
10X Tris-glycine-SDS	B46	1 L	25 mM Tris 192 mM glycine 0.1% SDS pH 8.3	• Denaturing protein polyacrylamide gel electrophoresis (1).	• For each electrophoresis run fresh 1X buffer should be prepared. • Concentrated buffer should be diluted to a working 1X solution before use.
10X Tris-glycine	B47	1 L	25 mM Tris 192 mM glycine pH 8.3	• Native protein polyacrylamide gel electrophoresis. • Western blotting.	
10X Tris-tricine-SDS	B48	1 L	100 mM Tris 100 mM tricine 0.1% SDS pH 8.3	• Denaturing polyacrylamide gel electrophoresis of low molecular weight proteins and peptides (2).	

Related Products

- ProteoJET™ Mammalian Cell Lysis Reagent p.392
- ProteoJET™ Cytoplasmic and Nuclear Protein Extraction Kit p.393
- Bradford Reagent, ready-to-use p.394
- Protein Standard Sets p.395
- Protein Standard Solution p.395
- Prestained Protein Ladders and Markers p.396
- Unstained Protein Ladders and Markers p.397
- PageSilver™ Silver Staining Kit p.398
- PageBlue™ Protein Staining Solution p.399
- DualColor™ Protein Loading Buffer Pack p.400
- Protein Loading Buffer Pack p.401
- DTT p.490

Quality Control

The absence of proteases, deoxyribonucleases and ribonucleases is confirmed by appropriate quality tests.

Storage

Store at room temperature.

References

1. Laemmli, U.K., Cleavage of structural proteins during the assembly of the head of Bacteriophage T4, Nature, 227, 680-685, 1970.
2. Shagger, H., and G. von Jagow, Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa, Anal. Biochem., 166, 368-379,1987.

Protocols and Recommendations

» SDS-PAGE Protocol p.404
» Semi-dry Protein Transfer for Western Blotting p.407
» Troubleshooting Guide p.408

Bulk quantities and custom formulations available upon request

General Recommendations for Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

- Low percentage gels are recommended for analysis of large proteins and high percentage gels for analysis of small proteins.
- Linear gradient gels allow for high resolution of a broad range of both small and large proteins.

Table 8.1. Gel Recommendations.

Protein MW range, kDa	Recommended gel, %
~5-50	18
~5-60	16
~10-80	14
~20-150	12
~30-200	10
~40-250	8
~60-300	6
~100-400	4
Protein MW range, kDa	**Recommended gradient gel, %**
~5-100	10-20
~5-300	4-20
~10-200	8-16
~30-300	4-12

- All Fermentas protein ladders/markers can be used on 6, 8, 10, 12, 14 % SDS polyacrylamide gels and on 4-12%, 8-16%, 4-20% and 10-20% gradient gels.
- The following general rule can be applied to protein ladders/markers as well as to protein samples:
  - in low percentage gels (4-8%), small proteins (10-15 kDa) migrate with the tracking dyes during electrophoresis and may be not visible;
  - in high percentage gels (14-18%) large proteins (150-250 kDa) may not separate.
- For more precise determination of molecular weights, unstained protein ladders/markers are recommended.
- Prestained standards are ideal for monitoring the process of electrophoresis and the protein transfer efficiency in Western blotting.
- Prestained proteins may have different mobilities in various SDS-PAGE buffer and gel systems due to coupled chromophores that affect protein mobility. Prestained standards are recommended when approximate sizing is enough.
- Each lot of prestained protein ladder/marker is calibrated against a precisely sized unstained protein ladder/marker in Tris-glycine-SDS gel and the calculated apparent molecular weights are reported in the product's Certificate of Analysis. The prestained protein may have different mobility in other electrophoresis buffer and gel systems. The migration pattern of a particular protein standard on various gel types is provided at www.fermentas.com.
- Modifications to native proteins such as phosphorylation and glycosylation may alter protein mobility. The molecular weights of modified proteins may not correspond to those of unmodified proteins of the same size.

Protein Ladder/Marker: Recommendations for Loading

Step	Protein ladder/marker	
	Spectra™ Multicolor Broad Range Protein Ladder (#SM1841) **PageRuler™ Plus Prestained Protein Ladder** (#SM1811) **PageRuler™ Prestained Protein Ladder** (#SM0671) **PageRuler™ Unstained Protein Ladder** (#SM0661) **Prestained Protein Molecular Weight Marker** (#SM1811)	**Unstained Protein Molecular Weight Marker** (#SM1811)
Thaw	Thaw the ladder either at room temperature or at 37°C for a few minutes to dissolve precipitated solids.	
Mix	Mix gently, but thoroughly, to ensure that the solution is homogeneous.	
Heat	Do not heat	Transfer the required aliquot to a clean tube with a screw cap. Heat at 95°C for 10 minutes. Cool and mix. Once denatured the marker can be further used just after the thawing.
Load for gels to be stained with PageBlue™ Protein Staining Solution (#R0571) or other Coomassie dyes, and for Western Blots	5 µl per well for mini-gels with a thickness of 0.75-1 mm. 10 µl per well for large gels with a thickness of 0.75-1 mm. ***Note***. Load 10 / 20 µl of Spectra™ Multicolor Broad Range Protein Ladder accordingly. For thicker gels, the loading volume should be increased.	
Dilution for gels to be stained with PageSilver™ Silver Staining Kit (#K0681) or other Silver staining technique	To avoid overloading the gel which will be subsequently silver stained, dilute the ladder/marker just prior to use: Water, nuclease-free (#R0581) 36.5 µl 5X Protein Loading Buffer (#R0891)* 10 µl 20X Reducing Agent (#R0891) 2.5 µl Protein ladder/marker 1 µl * Alternatively 4X DualColor™ Protein Buffer Loading Pack (#R1011) can be used. Volumes of the buffer and water should be adjusted appropriately. ***Note***. Heat Unstained Protein Molecular Weight Marker (#SM0431) as described above. Load 5 µl of the mixture on SDS polyacrylamide gel.	
Staining note	Staining is not required to visualize prestained protein ladders/markers. To visualize unstained protein ladders/markers, the gel can be processed with PageBlue™ Protein Staining Solution (#R0571), PageSilver™ Silver Staining Kit (#K0681) or other protein staining techniques.	

Protein Samples: Extraction and Quantification Guides

Sample/procedure	Extraction	Quantification
Mammalian cell and tissue samples	Extract total proteins using ProteoJET™ Mammalian Cell Lysis Reagent (#K0301) Extract nuclear and cytoplasmic proteins using ProteoJET™ Cytoplasmic and Nuclear Protein Extraction Kit (#K0311)	• Use Bradford Reagent, ready-to-use (#R1271) for protein quantification. • Easily create standard curves by using one of our convenient protein standard sets: – Bovine Serum Albumin Standard Set (#R1281), – Bovine Gamma Globulin Standard Set (#R1291), – Protein Standard Solution (#R0882). • Create a standard curve by plotting the 595 nm values (y-axis) versus their concentration in µg/ml (x-axis). • Use the standard curve to determine the protein concentration of each unknown sample. Refer to product certificates of analysis or www.fermentas.com for complete protocols.
Bacterial samples	To analyze total bacterial proteins in SDS-PAGE, cells can be treated directly with DualColor™ Protein Loading Buffer Pack (#R1011) or Protein Loading Buffer Pack (#R0891) as described on p.404.	
Lyophilized proteins	To analyze proteins in SDS-PAGE, treat lyophilized proteins directly with DualColor™ Protein Loading Buffer Pack (#R1011) or Protein Loading Buffer Pack (#R0891) as described on p.404.	

Protein Samples: Preparation for Loading on SDS-PAGE

Step	Sample preparation with the DualColor™ Protein Loading Buffer Pack (#R1011, p.400)	Sample preparation with the Protein Loading Buffer Pack (#R0891, p.401)
Thaw	Thaw the buffer pack components either at room temperature or at 37°C for a few minutes to dissolve precipitates.	
Mix	Vortex gently, but thoroughly to ensure that the solution is homogeneous.	
	2.0 µl of 20X Reducing Agent	2.5 µl of 20X Reducing Agent
	Protein sample (~0.5 ng – 2.5 µg) For Western blots or gels to be treated with Coomassie based stains, use up to 2.5 µg of total protein per minigel well. For silver staining applications use up to 10 ng of total protein per minigel well.	
	10 µl of 4X DualColor™ Protein Loading Buffer Water, nuclease-free (#R0581) to 40 µl*	10 µl of 5X Protein Loading Buffer Water, nuclease-free (#R0581) to 50 µl*
Denaturation	Incubate samples at 95-100°C for 5 minutes.	
Loading	Spin for a few seconds in a microcentrifuge. Apply directly to an SDS-polyacrylamide gel. Use ~10 µl per minigel well.	

* The sample volume can be scaled up or down.

SDS-PAGE

SDS Polyacrylamide Gel Electrophoresis Protocol

I. Reagents

30% acrylamide/bisacrylamide (37.5:1) aqueous solution (stored in the dark)
1.5 M Tris-HCl buffer (pH 8.8)
0.5 M Tris-HCl buffer (pH 6.8)
10% ammonium persulfate (APS) solution (always should be prepared freshly)
TEMED
1X Tris-glycine-SDS Buffer (#B46) (10X buffer diluted to 1X concentration prior use)

Caution. Acrylamide is a neurotoxin. Always wear gloves, safety glasses, and a surgical mask when working with acrylamide powder.

II. Resolving Gel Preparation

Components	Volume: 10 ml resolving gel solution (for 2 minigels)		
	for 8% gel	for 10% gel	for 12% gel
Deionized water	4.73 ml	4.13 ml	3.43 ml
30% acrylamide/bisacrylamide	2.7 ml	3.3 ml	4.0 ml
1.5 M Tris-HCl containing 0.4% SDS, pH 8.8	2.5 ml	2.5 ml	2.5 ml
10% APS	60 µl	60 µl	60 µl
TEMED	13 µl	13 µl	13 µl

III. Stacking Gel Preparation

Components	Volume: 5 ml stacking gel solution (for 2 minigels)
Deionized water	3.0 ml
30% acrylamide/bisacrylamide	700 µl
0.5 M Tris-HCl containing 0.4% SDS, pH 6.8	1.25 ml
10% APS	25 µl
TEMED	20 µl

IV. Procedure

1. Assemble the glass plate sandwich. Prepare the resolving gel solution as described above.
2. Add APS and TEMED last, mix carefully to avoid formation of bubbles.
   Important Note. Polymerization begins as soon as APS is added to the mixture, so all subsequent actions must be performed promptly.
3. Pour the gel solution between the glass plates with a pipette, leave about 1/4 of the space free for the stacking gel. Carefully cover the top of the resolving gel with 50% isopropanol, 0.1% SDS solution or water, and wait until the resolving gel polymerizes (~30 min). A clear line will appear between the gel surface and the solution on top when polymerization is complete.

(continued on next page)

4. Discard the water, isopropanol or SDS solution. Wash gently with double-distilled water.
5. Pour the stacking gel solution (prepared as described above, add APS and TEMED last) carefully with a pipette to avoid formation of bubbles.
   Important Note. Polymerization begins as soon as APS is added to the mixture, so all subsequent actions must be performed promptly.
6. Insert combs. Allow the gel to polymerize for at least 60 min.
7. Remove combs carefully. Put the gel into the electrophoresis tank, fill the tank (bottom and top reservoirs) with fresh 1X Tris-glycine-SDS Buffer, make sure that the gel wells are covered with the buffer.
8. Load protein ladder/marker and probes prepared as described on p.403.
9. Set an appropriate voltage and current depending on how many gels you run. Increase the power when the dye front reaches the running gel. For exact values refer to the table below:

Gel	1 minigel	2 minigels
Stacking gel (upper)	13 mA	25 mA
Resolving gel (lower)	25 mA	50 mA

Values presented are for 0.75 mm gels. For thicker gels the current should be appropriately increased.

10. Stop the electrophoresis run when the dye front reaches the bottom of the gel. Disassemble the gel sandwich and proceed with gel staining or Western blot procedures as described on p.407.

Gel Staining

Staining Procedure with PageBlue™ Protein Staining Solution (#R0571)

	With microwaving (fast protocol)	Without microwaving (conventional protocol)
Total time	25 min for native gels 40 min for SDS-containing gels	65 min for native gels 95 min for SDS-containing gels
1. Washing in water*		
	• Add 100 ml water • Microwave for 1 min • Wash with gentle agitation for 5 min • Discard the water } x 3	• Add 100 ml water and rinse • Discard the water } x 3
2. Staining		
	• Add 20 ml PageBlue™ Protein Stainin Solution • Microwave for 30 s • Stain with gentle agitation for 20 min • Discard the solution	• Add 20 ml PageBlue™ Protein Staining Solution • Stain with gentle agitation for 60 min (or overnight) • Discard the solution
3. Washing in water		
	• Add 100 ml water and rinse for 5 min	• Add 100 ml water and rinse for 5 min

*– only for SDS-containing gels

Note

- PageBlue™ Protein Staining Solution can be reused up to 3 times without a decrease in sensitivity.
- All reagent volumes are for 8x10 or 10x10 cm minigels of 0.75-1 mm thickness. Gels should be completely immersed in solution.
- When several gels are being stained, increase the amount of staining solution accordingly.
- The first wash step is crucial to remove SDS from the gel as SDS interferes with the staining reaction.
- For staining native gels without SDS, the washing step is not required.
- Staining sensitivity can be increased if the proteins are fixed for 15min either with 12% trichloracetic acid or with 25% isopropanol supplemented with 10% acetic acid. Fixation prevents protein diffusion from the gel and accelerates SDS removal. After fixation, gels can be stained immediately without additional washing.
- Using either the fast or conventional protocol, staining sensitivity is 5 ng of protein per band. To increase sensitivity to 0.05 ng per band the gel can be stained using the PageSilver™ Silver Staining Kit (#K0681) according to the recommended protocol on p.407.
- For staining peptides or small proteins (<10 kDa) fixation of the proteins for 15 min either with 12% trichloracetic acid or with 25% isopropanol supplemented with 10% acetic acid is recommended. Fixation prevents protein diffusion from the gel and accelerates SDS removal. After fixation, gels can be stained immediately without additional washing. Overnight staining time is required for peptide detection.

Staining Procedure with PageSilver™ Silver Staining Kit (#K0681)

	With microwaving (fast protocol)	Without microwaving (conventional protocol)
Total time	1 hour	2 hours 40 min
Sensitivity	0.1 ng/band	0.05 ng/band
1. Gel fixing 1		
	• Rinse the gel with deionized water • Add gel fixing solution 1 • Microwave for 30 s. Do not boil • Fix with gentle agitation for 10 min • Discard the solution	• Rinse the gel with deionized water • Add gel fixing solution 1 and gently agitate for 60 min • Discard the solution
2. Gel fixing 2 and washing		
	• Add gel fixing solution 2 • Microwave for 30 s. Do not boil • Fix with gentle agitation for 10 min • Discard the solution } x 2 • Add deionized water and gently agitate for 20 s • Discard the water } x 2	• Add gel fixing solution 2 and gently agitate for 20 min • Discard the solution } x 3 • Add deionized water and gently agitate for 20 s • Discard the water } x 2
3. Sensitizing and washing		
	• Add sensitizing solution and gently agitate for 1 min • Discard the solution • Add deionized water and gently agitate for 20 s • Discard the water } x 2	• Add sensitizing solution and gently agitate for 1 min • Discard the solution • Add deionized water and gently agitate for 20 s • Discard the water } x 2
4. Staining and washing		
	• Add staining solution and gently agitate for 20 min • Discard the solution • Add water and gently agitate for 20 s • Discard the water } x 2	• Add staining solution and gently agitate for 20 min • Discard the solution • Add water and gently agitate for 20 s • Discard the water } x 2
5. Developing		
	• Add developing solution and gently agitate for ~4 min • Discard the solution	• Add developing solution and gently agitate for 5-10 min • Discard the solution
6. Terminating		
	• Add stop solution and gently agitate for 5 min • Discard the solution	• Add stop solution and gently agitate for 10 min • Discard the solution

Note

For a complete protocol and troubleshooting, refer to the product manual or www.fermentas.com.

Protein Transfer

Semi-dry Protein Transfer for Western Blotting

Note. Wear gloves throughout the procedure to avoid contamination. Use 100 ml of each solution for mini gels (8x10 cm; 10x10 cm), for larger gels use enough of the solution to completely cover the gel/membrane/paper sheets in each step.

Buffers

Tris-glycine-methanol protein transfer buffer. Cool at 4°C before use.

Component	Amount	Final concentration
10X Tris-glycine buffer (#B47)	10 ml	1X
Methanol	10 ml	10% (v/v)
Deionized water	to 100 ml	

CAPS buffer for electrotransfer of proteins onto PVDF for N-terminal sequencing. Cool at 4°C before use.

Component	Amount	Final concentration
10X CAPS (100 mM, pH 11.0)	10 ml	10 mM
Methanol	10 ml	10% (v/v)
Deionized water	to 100 ml	

10X CAPS. Store at 4°C.

Component	Amount	Final concentration
CAPS (3-[cyclohexylamino]-1-propanesulfonic acid)	2.21 g	100 mM
Deionized water	to 90 ml	
2N NaOH	titrate to pH 11.0 (~4 ml)	
Deionized water	to 100 ml	

Ponceau S staining solution (only freshly made staining solution should be used).

Component	Amount	Final concentration
Ponceau S	0.2 g	0.2% (w/v)
Glacial acetic acid	1 ml	1% (v/v)
Deionized water	to 100 ml	

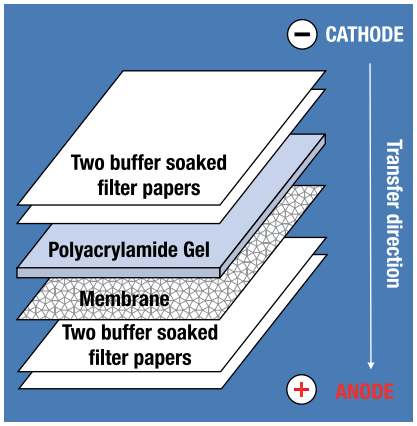


Figure 8.14. Blotting with a semi-dry transfer unit.

Semi-dry Protein Transfer

1. Presoak 2-4 pieces of blotting paper (cut to the size of the gel) in transfer buffer for 5 min.
2. Cut a piece of nitrocellulose membrane to the size of the gel and equilibrate it in transfer buffer. If a PVDF membrane is used, incubate it in methanol for 2 min before equilibrating it in transfer buffer. Use CAPS buffer for N-terminal sequencing, Tris-glycine-methanol protein transfer buffer is suitable for all other applications.
3. Carefully remove the stacking gel from the resolving gel. Soak the resolving gel in CAPS buffer for 5 min if this buffer is used. This step can be omitted if Tris-glycine-methanol protein transfer buffer is used.
4. Assemble the transfer sandwich with the resolving gel on the anode (+) as shown in Fig.8.14. Use one sheet of blotting paper or two pieces of filter paper on each side of the sandwich. Make sure all air bubbles are removed since they will affect the efficiency of the electroblotting.
5. Electrotransfer proteins from the gel on the membrane for ~60 min at room temperature. Maintain the current at 0.8 mA per 1 cm^2 of the gel area and limit the voltage to 15 V.
6. When the transfer is complete, turn off the power and peel off the layers of the sandwich until you reach the membrane. Remove the membrane with forceps and rinse it in deionized water.

Monitoring the Protein Transfer

The efficiency of electrotransfer can be monitored using prestained protein ladders (Spectra™ Multicolor Broad Range Protein Ladder (#SM1841), PageRuler™ Prestained Protein Ladder (#SM0671), PageRuler™ Plus Prestained Protein Ladder (#SM1811) and Prestained Protein Molecular Weight Marker (#SM0441)). The use of the DualColor™ Protein Loading Pack (#R1011) also allows for monitoring of Western blot protein transfer from gel to membrane. Alternatively, the extent of protein transfer can be determined by staining the polyacrylamide gel after the transfer or by staining the protein directly on the membrane. Proteins on PVDF membranes can be visualized with the PageBlue™ Protein Staining Solution, while Ponceau S, India Ink or Amido Black are recommended for nitrocellulose and PVDF membranes.

Membrane Staining and Destaining

Staining PVDF Membrane with PageBlue™ Protein Staining Solution

Note. PVDF membrane must be air-dried before staining.

1. Add PageBlue™ Protein Staining Solution (#R0571) to cover the PVDF membrane. Agitate gently for 2 min.
2. Wash with 30% ethanol with gentle agitation for 5 min.

To completely remove the stain, wash the membrane with the mixture of 30% acetonitrile and 20% ethanol for 5 min.

Troubleshooting Guide for Protein Electrophoresis & Analysis

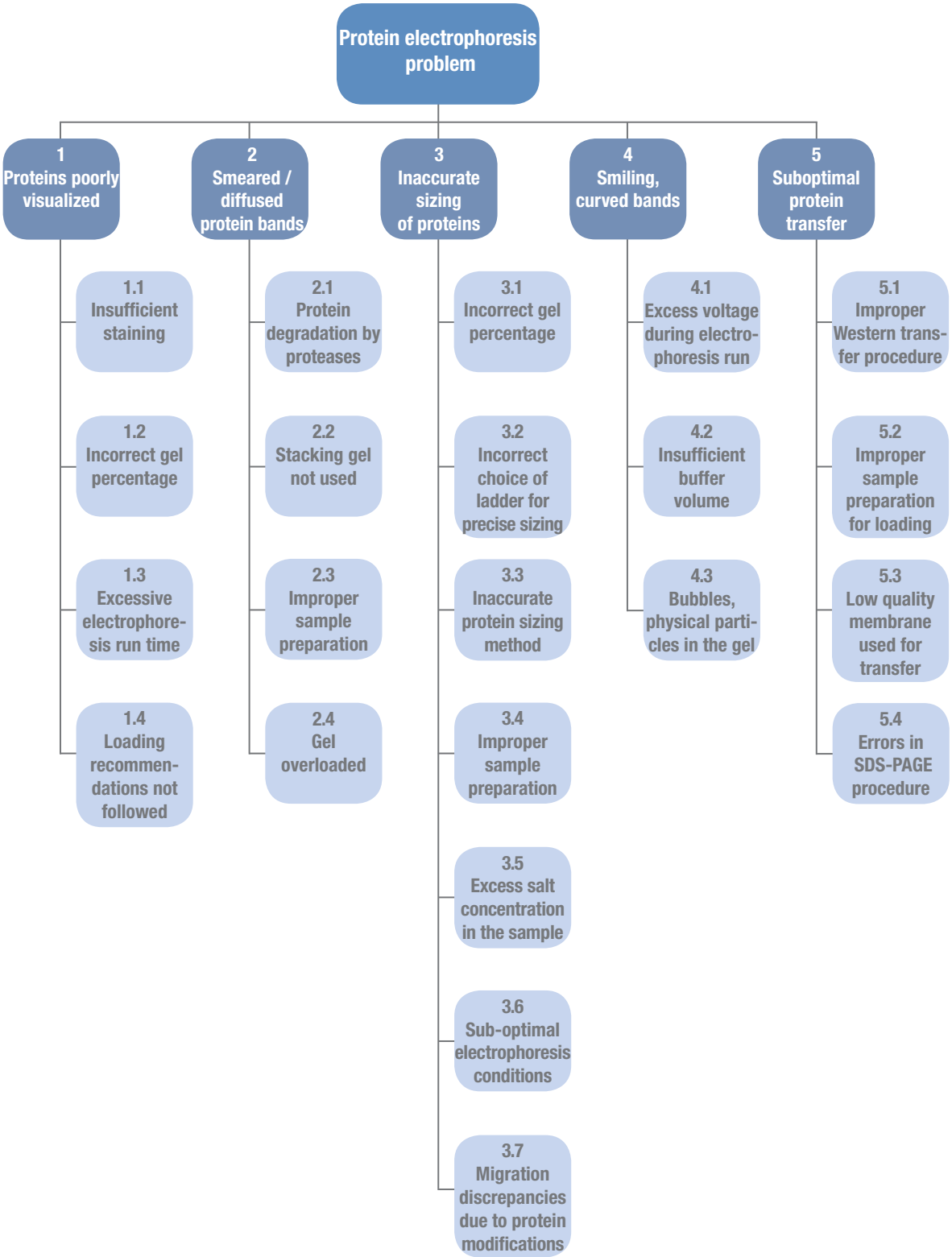


Table 8.2. Troubleshooting Guide for Protein Electrophoresis & Analysis.

Problem	Possible cause and recommended solution
1. Proteins poorly visualized	**1.1. Insufficient staining.** For Coomassie based staining, load ~0.5-5 µg of total protein per minigel well. 10 µg of total protein maybe required for lysates, 1-3 µg should be used to assay homogeneous protein. The staining sensitivity with PageBlue™ Protein Staining Solution (#R0571) is ~5 ng per band. Follow the protocol outlined in the manual. For silver staining procedures load ~0.1-2 ng of total protein per minigel well. The staining sensitivity with PageSilver™ Silver Staining Kit (#K0681) is ~0.05-0.6 ng per band. Unstained protein ladder/marker can be visualized with PageBlue™ Protein Staining Solution (#R0571), PageSilver™ Silver Staining Kit (#K0681) or other protein staining techniques.
	1.2. Incorrect gel percentage. Linear gradient gels allow for adequate resolution of both small and large proteins. Homogeneous low percentage gels are recommended for analysis of large proteins and high percentage gels for analysis of small proteins. In high percentage gels (14-18%) large proteins (150-250 kDa) may not separate, while in low percentage gels (4-8%) small proteins will migrate with a tracking dye. To choose the correct gel percentage for analysis of particular MW proteins, refer to the Table 8.1 on p.402.
	1.3. Excessive electrophoresis run time. Stop the electrophoresis run as soon as the tracking dye front reaches the bottom of the gel. In low percentage gels (4-8%), small proteins (10-15 kDa) migrate with the tracking dye during electrophoresis and may be not visible. Use high percentage or gradient gels to resolve low molecular weight proteins. To choose the right gel percentage for analysis of particular MW proteins, refer to the Table 8.1 on p.402.
	1.4. Loading recommendations not followed. Follow loading recommendation on p.403. Heat protein probes and Unstained Protein Molecular Weight Marker (#SM0431) as described. Do not heat other Fermentas protein ladders/marker.
2. Smeared/diffused protein bands	**2.1. Protein degradation by proteases.** Use clean tips and vials when handling proteins. Use protease inhibitors when extracting proteins. Store protein samples, ladders and markers at -20ºC.
	2.2. Stacking gel not used with the resolving gel. Placement of the stacking gel on top of the resolving gel is necessary to concentrate protein samples and to ensure accurate migration and separation into sharp bands. Follow the gel preparation recommendations on p.402.
	2.3. Improper sample preparation. To ensure proper migration during electrophoresis, protein samples should contain SDS, dithiothreitol (DTT) or 2-mercaptoethanol and must be heated prior to loading. Follow the recommendations for protein sample preparation and for protein ladders/markers on pp.403-404.
	2.4. Gel overloaded. For Coomassie based stains and Western blot applications use 0.5-5 µg of total protein per minigel well. For silver staining procedures use 0.1-2 ng of total protein per minigel well and dilute Fermentas protein ladder/marker 50 times just prior to use, *see* p.403.
3. Inaccurate sizing of proteins	**3.1. Incorrect gel percentage.** Use high percentage gels for analysis of small proteins and low percentage gels for analysis of large proteins. A gradient gel is ideal for precise determination of protein molecular weights. Refer to the table on p.402 to identify the correct percentage gel for a particular protein size.
	3.2. Incorrect choice of ladder/marker for precise sizing. For precise determination of molecular weights, only unstained protein ladders/markers should be used. Prestained standards are recommended only for approximate protein sizing, as chromophores that are covalently coupled to the prestained proteins affect their mobility in various SDS-PAGE-buffer and gel systems. However, they are suitable for approximate molecular weight determination when calibrated against unstained standards in the same system.

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Table 8.3. Troubleshooting Guide for Protein Electrophoresis & Analysis.

Problem	Possible cause and recommended solution
3. Inaccurate sizing of proteins	**3.3. Inaccurate protein sizing method.** Always create a standard curve based on the mobility of protein standards after digitizing a gel image. The standard protein mobility data can be used to prepare a graph of the relationship between the molecular weight of standard proteins and their relative mobility (R_f). Usually the functional relationship is calculated according to the formula log (MW) = a + b x R_f, where a and b are constants determined by calibration with known standards. The MW of an unknown protein is calculated by substituting its Rf in the equation outlined above. A new equation must be calculated for each gel, and data for several gels may be processed to create statistically robust results.
	3.4. Improper sample preparation. To ensure proper migration during electrophoresis, protein samples must contain SDS, dithiothreitol (DTT) or 2-mercaptoethanol and must be heated prior to loading. Follow recommendations for protein sample preparation and for protein ladders/markers on p.403.
	3.5. Excess salt concentration in the sample. High salt concentration in the sample will alter protein mobility. Remove excess salts by gel filtration.
	3.6. Suboptimal electrophoresis conditions from those used for ladder/marker calibration. The apparent molecular weights of Fermentas prestained protein standards are calibrated in classical Tris-glycine-SDS Laemmli system. Each lot of prestained protein ladder/marker is calibrated against a precisely sized unstained protein ladder/marker in Tris-glycine gel and the calculated apparent molecular weights are reported in the product's Certificate of Analysis and www.fermentas.com. However, the bands of the protein standard may have different mobilities in other electrophoresis buffer and gel systems. The migration pattern of a particular protein standard in different buffers and gels is provided on www.fermentas.com.
	3.7. Migration discrepancies due to protein modifications. Natural protein modifications such as; phosphorylation and glycosylation, may alter protein mobility. The molecular weights of modified proteins may or may not correspond to those of unmodified standard proteins of the same size.
4. Smiling, curved bands	**4.1. Excessive voltage during electrophoresis run.** Set the voltage to 250 V. Depending on a number of gels you run, use the appropriate power according the recommendations on p.405. Increase the power when dye front reaches the separating gel.
	4.2. Insufficient buffer volume. Fill the electrophoresis tank (bottom and top reservoirs) with fresh 1X Tris-glycine-SDS buffer, make sure that the gel wells are completely covered with buffer. Use cold buffer for electrophoresis.
	4.3. Bubbles, physical particles in the gel. Mix and pour all gel preparation solutions carefully to avoid formation of bubbles. If physical particles are visible in solutions, remove them by filtration.
5. Suboptimal protein transfer	**5.1. Improper Western transfer procedure.** Follow the recommendations for Western blot transfer. For semi-dry Western transfers follow the protocol on p.407. Make sure that buffer solutions completely cover the gel/membrane/paper sheets during all steps. Use unstained gels for transfer, as stained proteins are transferred with lower efficiency. Use prestained protein ladders (p.396) and the DualColor™ Protein Loading Buffer Pack (#R1011) for electrophoresis as they allow for monitoring of the transfer efficiency.
	5.2. Improper sample preparation for loading. To ensure proper migration during electrophoresis, protein samples must contain SDS, dithiothreitol (DTT) or 2-mercaptoethanol and must be heated prior to loading. Follow recommendations for protein sample preparation and for protein ladders/markers on pp.403-404.
	5.3. Low quality membrane used for transfer. Choose high quality PVDF membrane for Western blotting procedures. Low MW proteins are frequently transferred through nitrocellulose membranes and therefore may be not visible on the blot.
	5.4. Errors SDS-PAGE procedure. Follow specific recommendations for protein electrophoresis. Use prestained protein ladders (p.396) as they allow for monitoring of electrophoresis and transfer efficiency.