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.

RNA ELECTROPHORESIS

10

RNA Electrophoresis: Selection Guide

Introduction

RNA molecules can be analyzed on both native or denaturing agarose and polyacrylamide gels.

Non-denaturing RNA electrophoresis eliminates the need for hazardous chemicals, but due to intramolecular interactions, RNA molecules can form extensive double-stranded structures that are quite difficult to disrupt. As a result, accurate sizing of RNA molecules is not always possible under non-denaturing conditions. Native RNA electrophoresis is therefore typically used to asses the overall quality of total RNA.

Denaturing electrophoresis is recommended to precisely determine the size and integrity of RNA molecules.

Non-denaturing RNA Electrophoresis

(*see* protocol p.459)

Non-denaturing RNA electrophoresis can be performed using **50X TAE Buffer** (#B49) or **10X TBE Buffer** (#B52) and **TopVision™ LE GQ Agarose** (#R0491) gels.

Denaturing RNA Electrophoresis

(*see* protocols p.460)

Types of denaturing RNA electrophoresis conditions include:

- Denaturing polyacrylamide gel electrophoresis in TBE buffer supplemented with 7 M urea. The **RiboRuler™ Low Range RNA Ladder** (#SM1831/3) and 5% polyacrylamide-urea gel is recommended for analysis of smaller RNA molecules.
- Formaldehyde agarose gel electrophoresis in MOPS buffer. Formaldehyde forms unstable Schiff bases with the imino-groups of guanine residues. This maintains RNA in the denatured state.
- Glyoxal/DMSO agarose gel electrophoresis in sodium phosphate buffer. The two aldehyde groups of glyoxal react under slightly acid conditions with the imino-groups of guanine to form cyclic derivatives that maintain RNA in the denatured state.

Assessment of RNA Quality

After electrophoresis of total RNA samples in the presence of ethidium bromide, the 28S and 18S human rRNA should be clearly visible under UV illumination. Fast-migrating bands composed of 5.8S RNA and 5S RNA may also be visible depending on the RNA purification procedure. The intensity of the 28S RNA should be approximately twice the intensity of the 18S RNA. Smearing should not be visible around either band.

28S human rRNA migrates at approximately 5000 b while 18S human rRNA migrates at approximately 1900 b.

RNA Quantification

RiboRuler™ RNA ladders are suitable for in-gel RNA quantification. These ladders are produced from chromatography-purified RNA transcripts and are free from any degraded RNA or NTPs that often interfere with spectrophotometric measurements and lead to erroneous RNA quantification. Spectrophotometrically determined RNA concentrations of each ladder band are given on p.455.

Protocols and Recommendations on p.459 provides extensive information for successful RNA electrophoresis.

Troubleshooting Guide for RNA Electrophoresis provides solutions when problems arise.

For more information *see* www.fermentas.com.

Products for RNA electrophoresis:

- **RiboRuler™ Low Range RNA Ladder** (#SM1831/3), from 100 to 1000 b
- **RiboRuler™ High Range RNA Ladder** (#SM1821/3), from 200 to 6000 b

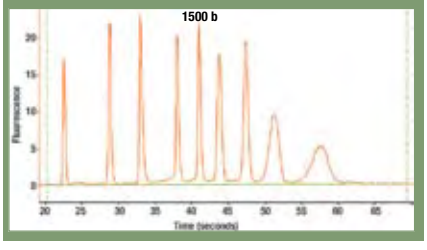
These ladders are mixtures of chromotography purified RNA transcripts that form sharp bands of uniform intensity and are ideal for ssRNA sizing on both native and denaturing gels.

RiboRuler™ RNA ladders are available in ready-to-use and conventional formats. Conventional versions can be labelled radioactively (*see* protocol on p.382) and are ideal for Northern blots.

- **2X RNA Loading Dye** (#R0641) is ideal for RNA probe and ladder preparation prior to electrophoresis. The solution contains an optimal concentration of formamide, which allows for separation of RNA molecules according to their size even under non-denaturing electrophoresis conditions. The presence of ethidium bromide in the solution allows for RNA visualization without additional staining of denaturing agarose gels.
- **50X TAE** (#B49) and **10X TBE** (#B52) buffers are free of RNases and are optimal for RNA gel electrophoresis. TAE buffer is recommended for analysis of larger RNA fragments, whereas TBE buffer is used for RNA molecules smaller than 1500 b and for denaturing polyacrylamide gel electrophoresis.
- **TopVision™ LE GQ Agarose** (#R0491) and **TopVision™ LM GQ Agarose** (#R0801) are ribonucleases-free and are recommended for analytical and preparative RNA electrophoresis.
- **GeneRuler™ and O'GeneRuler™ Ultra Low Range DNA Ladders** (#SM1211/2/3, #SM1223) can successfully be used for siRNA analysis (*see* Fig. 10.2 on p.456).

A

The exceptional purity of RiboRuler™ RNA ladders is confirmed by analysis on an **Agilent 2100 bioanalyzer**



B

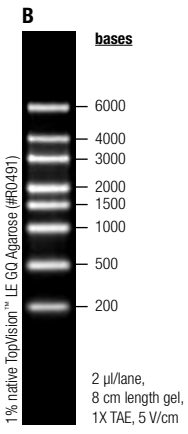


Figure 10.1. Analysis of RiboRuler™ High Range RNA Ladder (#SM1821), using an Agilent 2100 bioanalyzer, RNA 6000 Nano LabChip® Kit and agarose gel electrophoresis.
A – analysis of RiboRuler™ High Range RNA Ladder on Agilent 2100 bioanalyzer
B – agarose gel analysis of RiboRuler™ High Range RNA Ladder

Protocols and Recommendations

RiboRuler™ RNA Ladders (100- 6000 bases) -20°

Supplied with:
2X RNA Loading Dye 1 ml

For RNA sizing and quantification on native and denaturing gels.

RNA ladder	Catalog #	Volume, µl	Applications	Loading, µl/4-8 mm lane	Range, bases	Fragments	Agarose, %	PAGE, %
RiboRuler™ Low Range RNA Ladder	**SM1831**	100 (5x20)	50-100	1-2	100-1000	7	1.7-2.5	4.0-8.0
RiboRuler™ Low Range RNA Ladder, ready-to-use	**SM1833**	200 (5x40)		2-4				
RiboRuler™ High Range RNA Ladder	**SM1821**	100 (5x20)	50-100	1-2	200-6000	8	0.8-1.5	–
RiboRuler™ High Range RNA Ladder, ready-to-use	**SM1823**	200 (5x40)		2-4				

Related Products

- **TopVision™ Agaroses** p.458
- **2X RNA Loading Dye** p.456
- **50X TAE Buffer** p.457
- **10X TBE Buffer** p.457
- **Agarase** p.348
- **T4 Polynucleotide Kinase** p.246
- **Calf Intestine Alkaline Phosphatase** p.245
- **PageSilver™ Silver Staining Kit** p.398
- **RiboLock™ RNase Inhibitor** p.258
- **Products for *in vitro* Transcription** p.357
- **Products for First Strand cDNA Synthesis** p.279
- **0.5 M EDTA, pH 8.0** p.491
- **DEPC-treated Water** p.490

Description

RiboRuler™ RNA ladders are mixtures of chromatography purified single-stranded RNA transcripts, produced from specific templates that contain a fragment of the pTZ19R polylinker and Lambda phage fragments.

The RiboRuler™ High Range RNA Ladder is a mixture of 8 single-stranded RNA transcripts of 200 to 6000 b. The RiboRuler™ Low Range RNA Ladder is a mixture of 7 single-stranded RNA transcripts of 100 to 1000 b long. Each ladder is available in 1 mM EDTA, pH 6.0 or in a ready-to-use format, premixed with loading dye.

RiboRuler™ RNA ladders are composed of chromatography-purified transcripts what makes them free from NTPs and RNA degradation products. The concentration of each RNA transcript is determined spectrophotometrically (*see* photos below for precise quantities). To minimize the possibility of contamination during the use the ladders are aliquotted in smaller volumes.

RiboRuler™ RNA ladders (except the ready-to-use versions) are optimal for end-labeling using T4 Polynucleotide Kinase (#EK0031), *see* protocol on p.382, and are ideal for Northern blots.

Features

- Sharp bands of uniform intensity.
- Easy-to-remember band sizes and quantities.
- Predetermined concentration of ladder bands allows for approximate RNA quantification.
- Available in both conventional and ready-to-use format.
- Supplied with 2X RNA Loading Dye.
- Stable for 6 months at -20°C.

Applications

- RNA sizing and quantification on native or denaturing gels.
- Northern blotting.

Storage Buffer

1 mM EDTA (pH 6.0).

Storage and RNA Loading Buffer

(for ready-to-use ladders)
47.5% formamide, 0.0125% SDS, 0.0125% bromophenol blue, 0.0125% xylene cyanol FF, 0.0125% ethidium bromide and 0.75 mM EDTA.

2X RNA Loading Dye Solution

95% formamide, 0.025% SDS, 0.025% bromophenol blue, 0.025% xylene cyanol FF, 0.025% ethidium bromide and 0.5 mM EDTA.

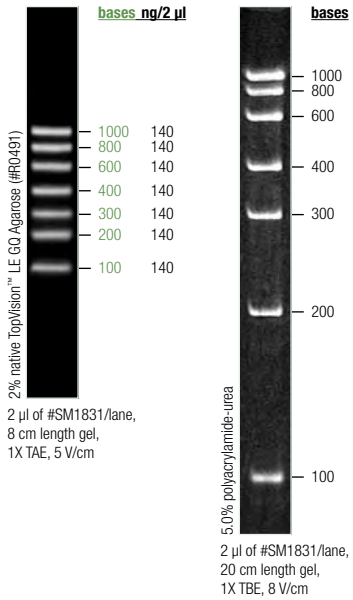
Quality Control

Tested in gel electrophoresis.
RNA concentration is determined spectrophotometrically. The absence of ribonucleases confirmed by appropriate tests.

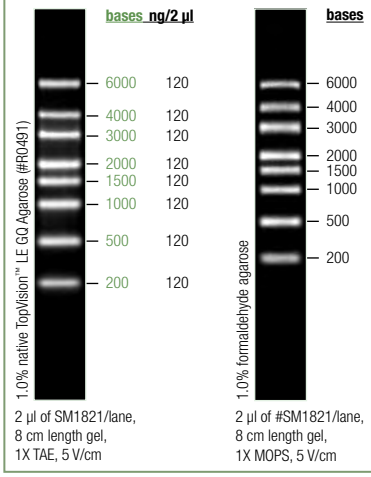
Storage

Store at -20°C for 6 months or at -70°C for longer periods.

RiboRuler™ Low Range RNA Ladder, #SM1831/3



RiboRuler™ High Range RNA Ladder, #SM1821/3

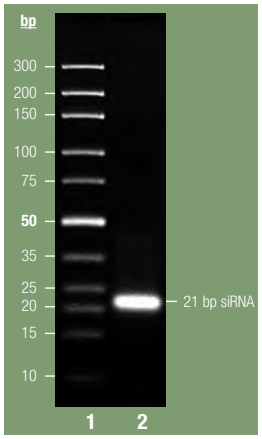


Protocols and Recommendations

» **General Recommendations** p.459
» **Preparation of RNA Ladder/Sample for RNA Electrophoresis** p.459
» **Preparation of:**
– **non-denaturing agarose gel** p.459
– **denaturing formaldehyde gel** p.460
– **denaturing glyoxal/DMSO gel** p.460
– **denaturing polyacrylamide/urea gel** p.460
» **Troubleshooting Guide** p.461
» **Radioactive RNA Labeling** p.382

Bulk quantities and custom formulations available upon request

DNA Ladders for siRNA ANALYSIS	Catalog #	Concentration, µg/µl	Amount, µg	Applications, 0.5 µg/lane	Loading, µg(µl)/lane	Range, bp	Fragments	Agarose, %	PAGE, %	Page
GeneRuler™ Ultra Low Range	SM1211	0.5	50	50-100	0.5-1 (1-2)	10-300	11	4.5-5.0	8-10	423
	SM1212		250 (5x50)	250-500						
	SM1213	0.1	50	100	0.5-1 (5-10)					
O'GeneRuler™ Ultra Low Range	SM1223	0.1	50	100	0.5-1 (5-10)					



Application
- siRNA analysis.

Figure 10.2. siRNA gel analysis by GeneRuler™ and O'GeneRuler™ Ultra Low Range DNA ladders.
Electrophoresis conditions: 5% agarose gel, 1X TBE buffer, 7 V/cm, 45 min.
1 – GeneRuler™ Ultra Low Range DNA Ladder
2 – 21 bp siRNA (0.5 µg), premixed with 6X DNA Loading Dye (#R0611)

For product description *see* chapter **DNA Electrophoresis** on p.421

Reagents for RNA Electrophoresis

2X RNA Loading Dye

#R0641 1 ml

Related Products

- TopVision™ Agaroses p.458
- RiboRuler™ RNA Ladders p.455
- 50X TAE Buffer p.457
- 10X TBE Buffer p.457
- PageSilver™ Silver Staining Kit p.398
- Agarase p.348
- Products for *in vitro* Transcription p.357
- 0.5 M EDTA, pH 8.0 p.491
- DEPC-treated Water p.490

Description
2X RNA Loading Dye is recommended for preparation of RiboRuler™ RNA ladders and RNA samples for electrophoresis on agarose or polyacrylamide gels. It contains the tracking dyes bromophenol blue and xylene cyanol FF, as well as the intercalating dye ethidium bromide. In most denaturing agarose gel systems, bromophenol blue migrates slightly faster than human 5S rRNA, whereas xylene cyanol FF migrates slightly slower than 18S rRNA.
2X RNA Loading Dye contains the denaturing agent formamide which allows RNA fragments to separate according to size even during non-denaturing electrophoresis. Formamide also stabilizes RNA.

Composition of 2X Solution
- 95% formamide
- 0.025% SDS
- 0.025% bromophenol blue
- 0.025% xylene cyanol FF
- 0.025% ethidium bromide
- 0.5 mM EDTA

Quality Control
Tested in RNA sample preparation prior to agarose gel electrophoresis. The absence of ribonucleases is confirmed by appropriate tests.

Storage
Store at 4°C up to 12 months.
For longer periods, store at -20°C.

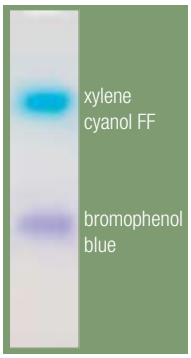


Figure 10.3. Electrophoresis of tracking dyes in 2X RNA Loading Dye.

Protocols and Recommendations

- General Recommendations p.459
- Preparation of RNA Ladder/Sample for RNA Electrophoresis p.459
- Preparation of:
  - non-denaturing agarose gel p.459
  - denaturing formaldehyde gel p.460
  - denaturing glyoxal/DMSO gel p.460
  - denaturing polyacrylamide/urea gel p.460
- Troubleshooting Guide p.461
- Radioactive RNA Labeling p.382

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

Electrophoresis Buffers

RT

Buffer	Cat #	Size	1X composition	Application and features	Usage recommendations
50X TAE Buffer (Tris-acetate-EDTA)	B49	1 L	40 mM Tris 20 mM acetic acid 1 mM EDTA pH of 50X TAE: 8.4	• Electrophoresis of nucleic acids in agarose and polyacrylamide gels. • Used both as a running buffer and as a gel preparation buffer. • Recommended for resolution of RNA and DNA fragments larger than 1500 b(p), for genomic DNA and for large supercoiled DNA. • Filtered through a 0.22 µm membrane.	• Use fresh 1X TAE both for the gel and for the electrophoresis run. • To prepare 1X TAE buffer, add 20 ml of 50X TAE buffer to 980 ml of deionized water and mix well. ***Note*** TAE buffer has a relatively low buffering capacity, therefore periodic replacement of the buffer during prolonged electrophoresis is recommended.
10X TBE Buffer (Tris-borate-EDTA)	B52	1 L	89 mM Tris 89 mM boric acid 2 mM EDTA pH of 10X TBE: 8.3	• Electrophoresis of nucleic acids in agarose and polyacrylamide gels. • Used both as a running buffer and as a gel preparation buffer. • Recommended for electrophoresis of RNA and DNA fragments smaller than 1500 b(p). • Filtered through a 0.22 µm membrane.	• Use fresh 1X TBE both for the gel and for the electrophoresis run. • To prepare 1X TBE buffer, add 100 ml of 10X TBE buffer to 900 ml of deionized water and mix well. ***Note*** Double-stranded linear nucleic acid molecules migrate about 10% slower in TBE buffer than in TAE buffer.

Related Products

- **TopVision™ Agaroses** p.458
- **RiboRuler™ RNA Ladders** p.455
- **2X RNA Loading Dye** p.456
- **PageSilver™ Silver Staining Kit** p.398
- **Agarase** p.348
- **DNA Markers/Ladders** p.411
- **DNA Loading Dyes** p.438
- **DNA Gel Extraction Kit** p.347
- **DEPC-treated Water** p.490

Quality Control

The absence of endo-, exodeoxyribonucleases and ribonucleases confirmed by appropriate quality tests.

Storage

The electrophoresis buffers can be stored indefinitely at room temperature. If the buffer is stored at lower temperatures, a precipitate may form, which is easily dissolved by gentle heating.

Protocols and Recommendations

» **General Recommendations** p.459
» **Preparation of RNA Ladder/Sample for RNA Electrophoresis** p.459
» **Preparation of:**
– **non-denaturing agarose gel** p.459
– **denaturing formaldehyde gel** p.460
– **denaturing glyoxal/DMSO gel** p.460
– **denaturing polyacrylamide/urea gel** p.460
» **Troubleshooting Guide** p.461

Bulk quantities and custom formulations available upon request

TopVision™ GQ Agarose

RT

Agarose	Cat #	Size	Characteristics	Features	Applications
TopVision™ LE GQ Agarose (low electroendosmosis)	R0491	100 g	• Electroendosmosis EEO 0.05-0.13 • Gel strength (1% gel) ≥1200 g/cm² • Gel strength (1.5% gel) ≥2500 g/cm² • Gelling temperature 36±1.5°C • Melting temperature 88±1.5°C • Moisture <7%	• Optimal concentration between 0.4-5% in all typical buffer systems. • GQ (Genetic Quality) certified, which ensures that nucleic acids recovered from preparative gels can be used for downstream applications (enzymatic reactions etc.). • Low DNA/RNA binding. • Excellent gel transparency. • DNase and RNase free.	• Analytical electrophoresis of nucleic acids. • Preparative electrophoresis. • Blotting assays.
TopVision™ LM GQ Agarose (low melting point)	R0801	25 g	• Electroendosmosis EEO <0.12 • Gel strength (1% gel) ≥250 g/cm² • Gelling temperature 24-28°C • Melting temperature <65.5°C • Moisture <7%	• Optimal concentration between 0.7-2% in all typical buffer systems. • GQ (Genetic Quality) certified, which ensures that nucleic acids recovered from preparative gels can be used for various applications (enzymatic reactions etc.). • Low DNA/RNA binding. • Excellent transparency of gels. • DNase and RNase free.	• In-gel enzymatic processing experiments. • Analytical electrophoresis of nucleic acids. • Preparative electrophoresis using Agarase (#EO0461).

Related Products

- 50X TAE Buffer p.457
- 10X TBE Buffer p.457
- RiboRuler™ RNA Ladders p.455
- 2X RNA Loading Dye p.456
- Agarase p.348
- DNA Markers/Ladders p.411
- DNA Loading Dyes p.438
- DNA Gel Extraction Kit p.347
- 0.5 M EDTA, pH 8.0 p.491
- DEPC-treated Water p.490

Quality Control

The absence of endo-, exodeoxyribonucleases and ribonucleases confirmed by appropriate quality tests.

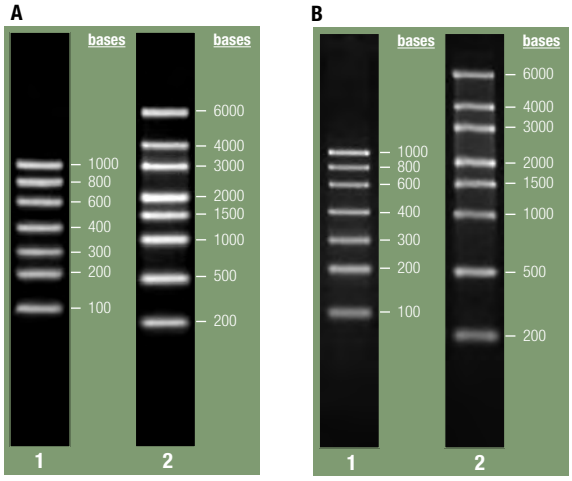


Figure 10.4. Separation of Fermentas RiboRuler™ RNA Ladders in (A) TopVision™ LE GQ Agarose, (B) TopVision™ LM GQ Agarose.
Electrophoresis conditions: 1X TAE buffer, 7 V/cm, 40 min.

1 – RiboRuler™ Low Range RNA Ladder, 2 µl/lane, 2% gel
2 – RiboRuler™ High Range RNA Ladder, 2 µl/lane, 1% gel

Protocols and Recommendations

» General Recommendations p.459
» Preparation of RNA Ladder/Sample for RNA Electrophoresis p.459
» Preparation of:
– non-denaturing agarose gel p.459
– denaturing formaldehyde gel p.460
– denaturing glyoxal/DMSO gel p.460
– denaturing polyacrylamide/urea gel p.460
» Troubleshooting Guide p.461
» Casting High Quality Agarose Gels p.253

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

Protocols and Recommendations

General Recommendations for RNA Electrophoresis

- RNA ladders, as well as any RNA, are extremely sensitive to degradation by ribonucleases. Use only fresh electrophoresis buffers and freshly poured gels.
- Use clean electrophoresis chambers. For RNA gel analysis, avoid electrophoresis tanks used for DNA miniprep analysis since DNA minipreps may contain RNase A or T1.
- Use the same loading dye for samples and for RNA markers. 2X RNA Loading Dye is available separately (#R0641) and is provided with all RiboRuler™ RNA ladders. It contains ethidium bromide for RNA visualization on denaturing formaldehyde gels. If RNA fragments are separated on native agarose gels or on polyacrylamide/urea gels, additional staining with ethidium bromide is recommended.
- For native gels, add 0.5 µg/ml of ethidium bromide to the agarose gel and to the running buffer.

Preparation of RNA Ladders and Samples for RNA Electrophoresis

Preparation of RNA Ladders for Electrophoresis

For RiboRuler™ RNA ladders (#SM1831 and #SM1821):

1. Mix 1 volume of RNA ladder and 1 volume of the supplied 2X RNA Loading Dye (#R0641).
2. Heat at 70°C for 10 min.
3. Chill on ice and spin down prior to loading on a gel.
4. Load 0.5 µl of the prepared ladder for every mm of gel lane width (4 µl / 8 mm lane).

For RiboRuler™ RNA ladders, ready-to-use (#SM1833 and #SM1823):

1. Heat RNA ladders at 70°C for 10 min.
2. Chill on ice for 3 minutes and spin down prior to loading on a gel.
3. Load 0.5 µl of the ladder for every mm of gel lane width (4 µl / 8 mm lane).

Note

Ladders prepared as described above are not suitable for glyoxal/DMSO agarose gel electrophoresis. To prepare ladders for glyoxal/DMSO agarose gels, please refer to the protocol on p.460.

Preparation of RNA Samples for Electrophoresis

1. Mix 1 volume of the 2X RNA Loading Dye (#R0641) and 1 volume of the RNA sample.
2. Heat at 70ºC for 10 min.
3. Chill on ice for 3 minutes and spin down prior to loading on a gel.

Note

RNA samples prepared as described above are not suitable for glyoxal/DMSO agarose gel electrophoresis. To prepare RNA samples for glyoxal/DMSO agarose gels, refer to the protocol on p.460.

Preparation of Gels for RNA Electrophoresis

Non-denaturing Agarose Gel

- Use a flask of at least three times larger volume than that of the solution to avoid boiling over.
- Use the same 1X electrophoresis buffer to prepare the gel and to run electrophoresis.
- Dilute 50X TAE (#B49) or 10X TBE (#B52) buffers to a 1X concentration immediately before use.
- Use TBE buffer for analysis of RNA bands smaller than 1500 b. For larger RNA, use TAE buffer.

1. Weigh out the required amount of agarose (depending on the gel %) into an Erlenmeyer flask.
2. Add the appropriate volume of either 1X TBE or 1X TAE buffer and swirl to mix.
3. Weigh the flask with the solution.

For high percentage gels (3-5%): *add an excess amount of distilled water to increase the weight by 10-20%.*

4. Boil the mixture in a microwave oven (at middle power) until the agarose melts completely; swirl the flask several times while boiling. To prepare the highest quality agarose gels of any percentage, an additional 3-5 min of boiling after completely melting the agarose is recommended. A significant amount of water evaporates during this procedure and therefore restoring of the initial weight (in step 5) is required to obtain the desired percentage gel.
5. Weigh the flask again and if necessary, add hot distilled water to restore the initial weight.

For high percentage gels (3-5%): *check (by weighing) that the excess 10-20% of water has evaporated and, if needed, continue boiling to remove any excess, or add hot distilled water to restore the initial weight.*

Optional. For intensified gel staining add ethidium bromide to a final concentration of 0.5 µg/ml. Mix well and heat for an 1 minute without boiling.

6. Cool the solution to 65-70°C. Pour carefully on a clean casting plate with an appropriate comb. If bubbles appear, push them carefully away to the sides with a pipette tip.
7. Solidify the gel for approximately 30 min before use. Low percentage LM agarose gels can be solidified at 4°C.
8. Immerse the gel into the desired electrophoresis buffer.

(continued on next page)

9. Heat the RNA samples and ladder at 70°C for 10 min, then chill on ice for 3 min. Load onto the gel.
10. Run electrophoresis at 5 V/cm until the bromophenol blue runs approximately two-thirds of the way down the gel.

After electrophoresis the gel can be stained by immersing it into a 0.5 µg/ml ethidium bromide solution for 20 min, stained with SYBR® Green II or any other RNA staining technique.

Warning. Hot agarose solution should be handled very carefully.

Denaturing Formaldehyde Gels in MOPS Buffer

1. Freshly prepare 10X MOPS buffer:
   0.4 M MOPS (pH 7.0),
   0.1 M sodium acetate,
   0.01 M EDTA (pH 8.0).
2. Prepare 1% TopVision™ LE GQ Agarose (#R0491) gel as follows:
   – stir 1g of agarose powder in 72 ml of deionized water;
   – melt the agarose, and then add 10 ml of 10X MOPS buffer and mix;
   – when the agarose solution cools to 60°C, add 18 ml of fresh formaldehyde (37%) in a fume hood and mix thoroughly;
   – pour the gel.
3. Place the gel into an electrophoresis apparatus containing 1X MOPS buffer.
4. Heat the RNA samples and ladder at 70°C for 10 min, and then chill on ice for 3 min (p.459).
5. Load onto the gel.

Note

There is no need to stain the gel as ethidium bromide present in 2X RNA Loading Dye is sufficient for visualization under UV light.

Denaturing Glyoxal/DMSO Gels in Sodium Phosphate Buffer

1. Prepare thick 1% TopVision™ LE GQ Agarose (#R0491) gel in 0.01 M sodium phosphate buffer, pH 7.0.
2. Place the gel into an electrophoresis apparatus with 0.01 M sodium phosphate buffer, pH 7.0.
3. Prepare for loading 25 µl aliquots of the ladder/samples by adding:

Glyoxal (40% solution)	4.5 µl
DMSO	12.5 µl
0.1 M sodium phosphate buffer, pH 7.0	2.5 µl

4. Mix and add:

RiboRuler™ RNA Ladder *or* **RNA sample**	3 µl
2X RNA Loading Dye	1 µl
DEPC-treated Water (#R0603)	to 25 µl

5. Incubate for 1 hour at 50°C and then cool down to room temperature.
6. Load the samples on a gel.
7. Run electrophoresis at 5 V/cm until the bromophenol blue runs approximately two-thirds of the way down the gel.
8. Stain the gel in ethidium bromide solution (final concentration 0.5 µg/ml) in 0.5 M ammonium acetate for 15-30 min.
9. Wash the gel in fresh 0.5 M ammonium acetate solution for 15-30 min.

Denaturing Polyacrylamide/urea Gels in TBE Buffer

1. Prepare 20 ml of a 5% polyacrylamide gel containing 7 M urea by adding:

47.5% acrylamide: 2.5% bis-acrylamide solution	2 ml
10M urea	14 ml
10X TBE Buffer (#B52)	2 ml
10% freshly prepared ammonium persulfate	0.2 ml
deionized water	1.8 ml

2. Mix and add 10 µl TEMED. Mix again and pour the gel carefully avoiding the formation of air bubbles.
3. Insert the comb into the acrylamide and allow the gel to polymerize for at least 1 hour.
4. Fill the electrophoresis apparatus with 1X TBE buffer.
5. Heat the RNA samples and ladder at 70°C for 10 min, and chill on ice for 3 min (p.459).
6. Load onto the gel.
7. Run electrophoresis at 8 V/cm for about 1 hour.
8. Soak the gel for about 15 minutes in 1X TBE to remove urea prior to staining.
9. Stain the gel in 0.5 µg/ml ethidium bromide in 1X TBE solution for 15 min.

Troubleshooting Guide for RNA Electrophoresis

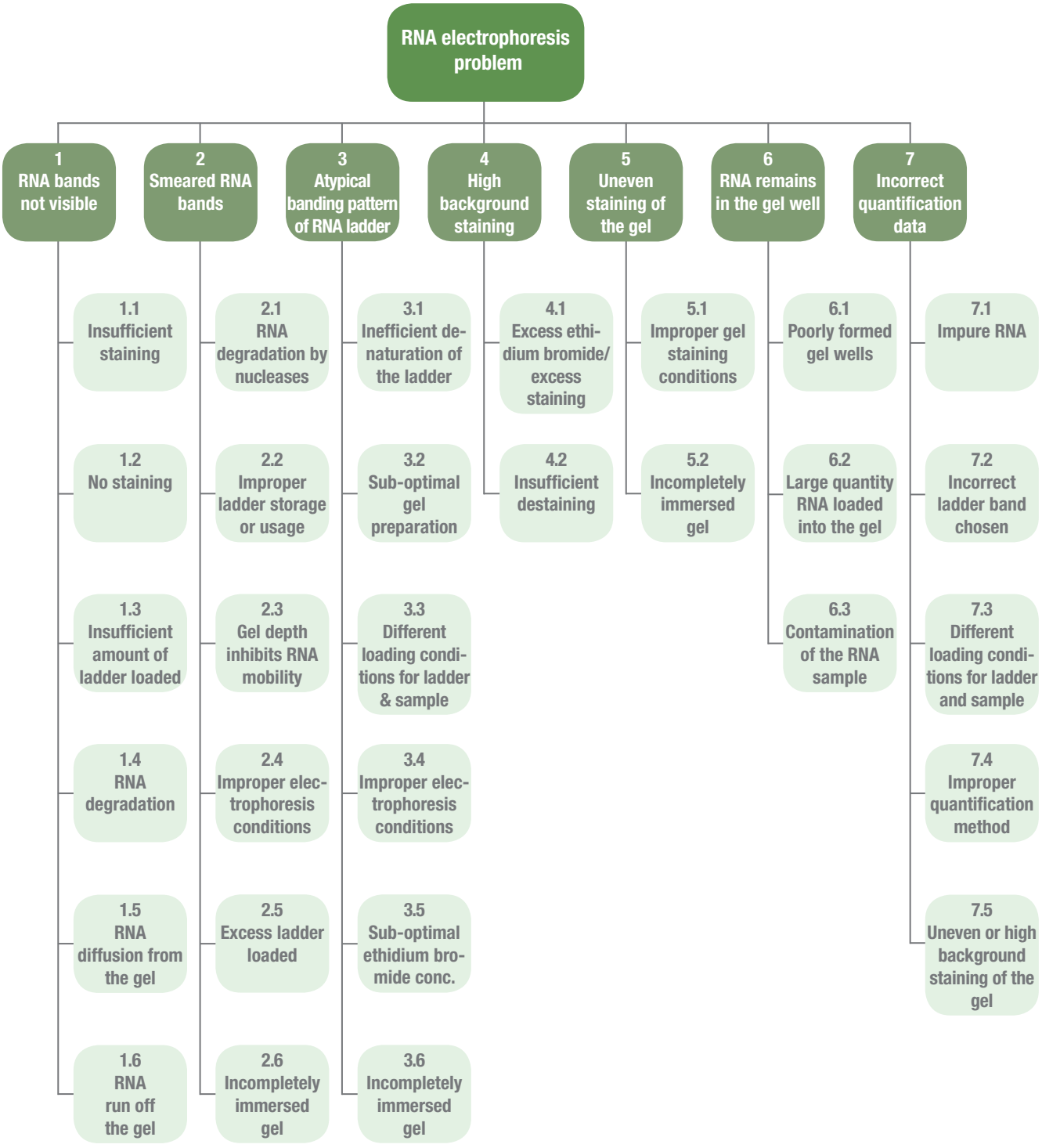


Table 10.1. Troubleshooting Guide for RNA Electrophoresis.

Problem	Possible cause and recommended solution
1. RNA bands are not visible	**1.1. Insufficient staining.** Use the 2X RNA Loading Dye for both conventional RiboRuler™ RNA ladder and RNA sample preparation prior to electrophoresis. This solution includes ethidium bromide at a concentration sufficient to stain RNA on denaturing formaldehyde agarose gels. Ready-to-use RiboRuler™ RNA ladders are premixed with 2X RNA Loading Dye. If RNA fragments are separated on native agarose gels, additional staining with ethidium bromide (final concentration 0.5 µg/ml) is recommended. If RNA is separated on a denaturing glyoxal/DMSO agarose gel, stain the gel in ethidium bromide solution (final concentration 0.5 µg/ml) in 0.5 M ammonium acetate for 15-30 min after electrophoresis. Wash the gel in a fresh 0.5 M ammonium acetate solution for 15-30 min. If RNA is separated on a denaturing polyacrylamide gel with urea, soak the gel for about 15 min in 1X TBE to remove the urea prior to staining. Stain the gel in 0.5 µg/ml ethidium bromide in 1XT BE solution for 15 min.
	1.2. No staining. If you are using loading dye which does not contain ethidium bromide, add ethidium bromide to both the agarose gel and electrophoresis buffer at a final concentration of 0.5 µg/ml. Alternatively, stain the gel after electrophoresis with ethidium bromide (0.5 µg/ml ethidium bromide) for 20 min, or SYBR® Green II (follow supplier recommendations).
	1.3. Insufficient amount of ladder was loaded. Follow the recommendations for loading described in the certificate of analysis of the RiboRuler™ RNA ladders (0.25 µl per mm gel lane for conventional ladders; 0.5 µl per mm gel lane for ready-to-use ladders).
	1.4. RNA degradation. Minimize exposure to UV light as this may cause RNA degradation/fading. RNA, including the RiboRuler™ RNA ladders, is extremely sensitive to degradation by ribonucleases. The use of fresh electrophoresis buffers, freshly poured gels, DEPC-treated solutions and protective gloves is recommended.
	1.5. RNA diffusion from the gel. Avoid prolonged electrophoresis or excessive staining and destaining procedures as this may cause diffusion of smaller RNA fragments from the gel. Avoid long term storage of the gel prior to photo documentation, as this may cause diffusion of RNA fragments and band fading.
	1.6. RNA has run off the gel. Stop electrophoresis after the bromophenol blue passes two thirds down the length of the gel. In most denaturing agarose gel systems, bromophenol blue migrates slightly faster than 5S rRNA and xylene cyanol FF migrates slightly slower than 18S rRNA. Make sure that the electrophoresis tank is in a completely vertical position.
2. Smeared RNA bands	**2.1. RNA degradation by nucleases.** RNA, including the RiboRuler™ RNA ladders, is extremely sensitive to degradation by ribonucleases. The use of fresh electrophoresis buffers, freshly poured gels, DEPC-treated solutions and protective gloves is recommended.
	2.2. Improper storage or use of RNA ladders. Store RiboRuler™ RNA ladders at -20°C for 6 months or at -70°C for 24 months. Thaw the ladders on ice.
	2.3. Excessive gel depth or sample volume. Use thin (~0.5 cm) gels and avoid loading of large volumes in the gel lane.
	2.4. Improper electrophoresis conditions. Ensure that there is enough electrophoresis buffer in the electrophoresis apparatus and that the gel is immersed completely. Do not use an excessively high voltage for electrophoresis. Run agarose gels at 5 V/cm (polyacrylamide/urea gels at 8 V/cm). To increase the band sharpness, use a lower voltage for several minutes at the beginning of electrophoresis. However, very low voltage during the entire run may result in band diffusion.
	2.5. Excessive RNA ladder loaded onto the gel. Follow the recommendations for loading described in the certificate of analysis of the RiboRuler™ RNA ladders (0.25 µl per mm gel lane for conventional ladders; 0.5 µl per mm gel lane for ready-to-use ladders).
	2.6. Incompletely immersed gel. Always ensure that there is enough electrophoresis buffer in the electrophoresis apparatus.

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Table 10.1. Troubleshooting Guide for RNA Electrophoresis.

Problem	Possible cause and recommended solution
3. Atypical banding pattern	**3.1. Inefficient denaturation of the ladder.** All RiboRuler™ RNA ladders should be heated to 70°C for 10 min, chilled on ice for 3 min and briefly centrifuged before loading on the gel in order to completely denature the RNA. Sample RNA should be prepared the same way.
	3.2. Sub-optimal gel preparation. Older formaldehyde has an acidic pH which may cause extra RNA bands on the gel. Use only fresh formaldehyde for optimal results.
	3.3. Different loading conditions for the sample and the ladder. Both ladder and sample RNA should be prepared with the same loading dye solution and loaded under the same conditions. After electrophoresis of total RNA samples in the presence of ethidium bromide, the 28S and 18S human rRNA should be clearly visible under UV illumination. Fast-migrating bands composed of 5.8S RNA and 5S RNA may also be visible depending on the RNA purification procedure. The intensity of the 28S RNA should be approximately twice the intensity of the 18S RNA. The 28S human rRNA band migrates at approximately 5000 b and the 18S human rRNA band migrates at approximately 1900 b.
	3.4. Improper electrophoresis conditions. Excessively long electrophoresis runs may result in migration of small RNA fragments off the gel. Very short electrophoresis runs may result in incompletely resolved bands. Run agarose gels at 5 V/cm (polyacrylamide/urea gels at 8 V/cm) until the bromophenol blue passes 2/3 of the gel length. TAE buffer is recommended for analysis of larger RNA, and TBE buffer is used to resolve RNA fragments smaller than 1500 b and for denaturing polyacrylamide gel electrophoresis. The correct gel percentage is important for optimal separation of the ladder RNA; take into account the following: RiboRuler™ High Range RNA Ladder (#SM1821/3) can be loaded on: – native 0.8-1.5% agarose gel with TAE buffer – denaturing formaldehyde 0.8-1.5% agarose gel with MOPS buffer – denaturing glyoxal/DMSO 0.8-1.5% agarose gel with sodium phosphate buffer RiboRuler™ Low Range RNA Ladder (#SM1831/3) can be loaded on: – native 1.7-2.5% agarose gel with TBE buffer – denaturing formaldehyde 1.7-2.5% agarose gel with MOPS buffer – denaturing glyoxal/DMSO 1.7-2.5% agarose gel with sodium phosphate buffer – denaturing 4-8% polyacrylamide gel with TBE buffer
	3.5. Sub-optimal ethidium bromide concentration in sample and ladder. The 2X RNA Loading Dye allows for RNA visualization without additional staining of denaturing agarose gels. Addition of extra ethidium bromide to the ladder or sample is not recommended and may result in RNA migration in the direction of the cathode. If RNA fragments are separated on native agarose gels or on polyacrylamide/urea gels, additional staining with ethidium bromide after electrophoresis is recommended.
	3.6. Incompletely immersed gel. Always ensure that there is enough electrophoresis buffer in the electrophoresis apparatus.
4. High background staining	**4.1. Excessively high ethidium bromide concentration or prolonged staining.** Use ethidium bromide at a final concentration of 0.5 µg/ml. Avoid prolonged staining of the gels.
	4.2. Insufficient gel destaining. If the gel is extensively stained with ethidium bromide, additional destaining in water is needed to remove background staining. Wash glyoxal/DMSO agarose gels after staining in a fresh 0.5 M ammonium acetate solution for 15-30 min.

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Table 10.1. Troubleshooting Guide for RNA Electrophoresis.

Problem	Possible cause and recommended solution
5. Uneven staining of the gel	**5.1. Improper gel staining conditions.** Ethidium bromide migrates in the opposite direction of the RNA during electrophoresis. Therefore, if ethidium bromide is only added to the agarose gel and not to the electrophoresis buffer, it may result in uneven RNA fragment staining. When 2X RNA Loading Dye is used for both conventional RiboRuler™ RNA ladders and RNA sample preparation prior to electrophoresis, additional staining is not required as the loading dye includes sufficient ethidium bromide to stain RNA on denaturing formaldehyde agarose gels. For native agarose gels, ethidium bromide (0.5 µg/ml) should be added to both the electrophoresis buffer and the agarose. This ensures an even distribution of ethidium bromide during electrophoresis so that the intensity of the bands upon exposure to UV light will be proportional to the quantity of RNA present.
	5.2. Incompletely immersed gel. Always ensure that there is enough electrophoresis buffer in the electrophoresis apparatus or enough of the staining solution during the staining so that the gel is always immersed completely.
6. RNA remains in the gel well	**6.1. Poorly formed gel wells.** Remove the gel comb only after complete polymerization of the gel. Pour the buffer onto the gel immediately. Rinse the wells with electrophoresis buffer to remove urea from denaturing polyacrylamide gels prior to loading the sample.
	6.2. Large quantity of RNA loaded into the gel. Follow the recommendations for loading described in the certificate of analysis of the RiboRuler™ RNA ladders (0.25 µl per mm gel lane for conventional ladders; 0.5 µl per mm gel lane for ready-to-use ladders).
	6.3. Contamination of the RNA sample. Make sure that your sample RNA solution does not contain any precipitate.
7. Incorrect quantification data	**7.1. Impure RNA.** Free NTPs and truncated transcripts remaining in the sample after *in vitro* transcription can interfere with spectrophotometrical measurements and lead to inaccurate quantification of sample RNA. RiboRuler™ RNA ladders are produced from chromatography-purified RNA transcripts and are free of any NTPs and truncated transcripts. Therefore the gel quantification data is compatible with the spectrophotometrical measurements of RiboRuler™ RNA ladders.
	7.2. Incorrect RiboRuler™ band chosen for quantification of the sample. Always compare the sample band with a similar size ladder band.
	7.3. Different loading conditions for the ladder and samples. Both sample and ladder RNA should be loaded under the same conditions. Use the supplied 2X RNA Loading Dye for the sample and ladder. Load equal volumes of sample RNA and ladder RNA. The required volume of sample RNA can be obtained by diluting with a mixture (1:1) of DEPC-treated Water (#R0603) and 2X RNA Loading Dye.
	7.4. Improper quantification method used. If possible, quantify by video-densitometry measurements while subtracting the gel background as this method is more precise than a visual comparison of the bands.
	7.5. Uneven staining of the gel and high background staining can also interfere with gel quantification results (*see* Problem 4 and 5 above).