Highest quality & performance

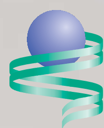
ISO 9001 ISO 14001

A New Standard in DNA Digestion

Innovative FastDigest™ Enzymes

Conventional Restriction Enzymes

...discover the benefits...



Fermentas
LIFE SCIENCES

P13

Fermentas – a Leader in Restriction Enzyme Research & Production

Fermentas, a leader in the field of restriction enzyme research and production, has more than 30 years of experience. Our scientists have discovered over 30% of all known restriction enzymes due to intensive screening and characterization of restriction-modifying enzymes. We have one of the largest collections of restriction enzyme-producing bacterial species in the world. Active screening for new restriction enzyme specificities is currently under way at Fermentas.

From the beginning, our main objective was to understand DNA restriction-modification (R-M) enzymes through an extensive enzyme screening and characterization program. Our research efforts have been directed towards:

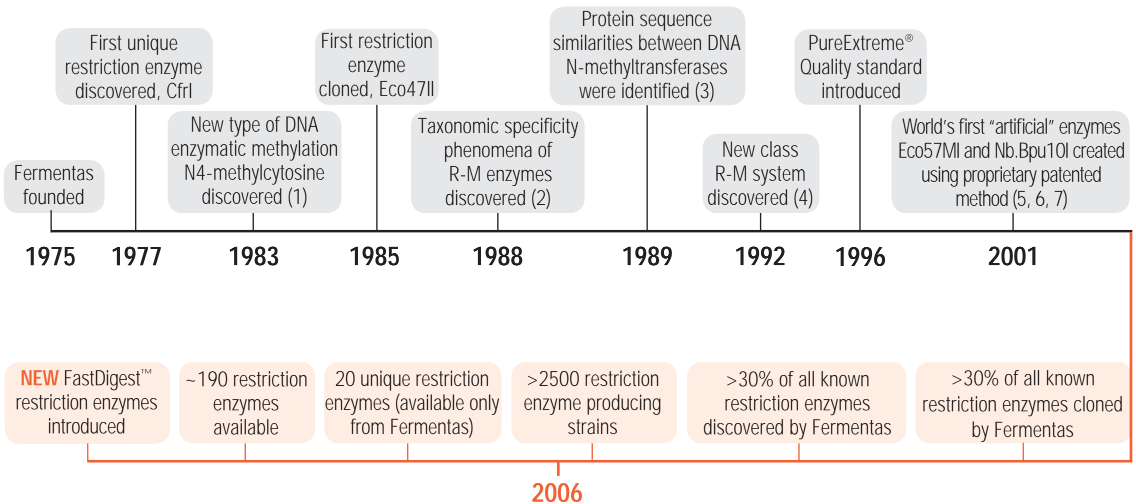
- Screening of new R-M enzymes
- Cloning the genes of R-M enzymes
- Investigation of their structural organization and mechanisms of expression
- Studies of the mechanisms of sequence discrimination by restriction enzymes and methyltransferases
- Rational design of enzymes with new specificities (*see* chart below, year 2001)

All of these efforts and knowledge have been integrated to create the FastDigest™ enzyme line – products that provide unrivalled convenience, speed and confidence for our customers.

Digestion of DNA with FastDigest™ enzymes

ONE reaction buffer • **ONE** reaction temperature • **ONE** µl of FastDigest™ enzyme • **ONE** µg of substrate DNA • **5 MINUTE** reaction

Fermentas Restriction Enzyme Facts



References

1. Janulaitis, A.E., Klimasauskas, S., Petrusyte, M., and Butkus, V., FEBS Lett., 161, 131-134, 1983.
2. Janulaitis, A.E., Kazlauskiene, R., Lazareviciute, L., Gilvonauskaite, R., Steponaviciene, D., Jagelavicius, M., Petrusyte, M., Bitinaite, J., Vezeviciute, Z., Kiuduliene, E., and Butkus, V., Gene, 74, 229-232, 1988.
3. Klimasauskas, S., Timinskas, A., Menkevicius, S., Butkiene, D., Butkus, V., and Janulaitis, A.A. Nucleic Acids Res., 17, 9823-9832, 1989.
4. Janulaitis, A., Petrusyte, M., Maneliene, Z., Klimasauskas, S., and Butkus, V., Nucleic Acids Res., 20, 6043-6049, 1992.
5. Rimseliene, R., Maneliene, Z., Lubys, A., Janulaitis, A., J Mol Biol., 327, 383-391, 2003
6. N.Bpu10I and the process of its preparation is covered by US patent No. 6867028 and corresponding counterparts.
7. Eco57MI and the process of its preparation is covered by US patent No. 6893854 and corresponding counterparts.

BENEFITS OF FERMENTAS RESTRICTION ENZYMES

Fermentas is committed to make your DNA digestion experiments as convenient, fast and robust as possible.

- PureExtreme® Restriction Enzyme Products – highest quality standard in the field:
  - Restriction enzymes free of contaminating activities (DNases, phosphatases)
  - Stringent quality control with the most advanced tests
  - ISO9001 & ISO14001 is your assurance of consistency and lot-to-lot reproducibility
  - The performance you need for your most demanding experiments
- Successful screening program for the discovery of enzymes with new specificities to create new research tools
- Complete line of user-friendly on-line tools has been generated to facilitate your experiments
- Comprehensive technical information has been collected and systematized to assist your research

Fermentas feature	Your benefits	Where to read more?
Premium line – FastDigest™ enzymes	• 5 minute digestion • One buffer system • One incubation temperature	pages 4-9 www.fermentas.com/fastdigest
188 conventional restriction enzymes	• 178 different sequence specificities • All common polylinker enzymes • All common enzymes for RFLP and AFLP genotyping	page 2 www.fermentas.com/research
20 unique restriction enzymes, available only from Fermentas	• 17 unique sequence specificities • 2 unique cleavage sites • 1 unique nicking enzyme	page 2 www.fermentas.com/uniquere
Active screening for new restriction enzyme specificities	• One of the largest collections of restriction enzyme producing bacterial strains (>2500) • Continuous release of new restriction enzymes to meet your current and future needs (~10 new enzymes per year)	page 2 www.fermentas.com/fastdigest www.fermentas.com/research
ISO9001 and 14001 certified primary manufacturer, unique quality control	• Guaranteed of PureExtreme® Quality for all restriction enzymes • Lot-to-lot reproducibility • Bulk quantities available	page 12 www.fermentas.com
30 years of experience	• Reliable supplier with solid expertise • Professional scientific support • Customized solutions for your special needs	page 2 www.fermentas.com

Contact Fermentas distributor in your country to order your Fermentas PureExtreme® restriction enzymes. Visit www.fermentas.com for the contact information.

FastDigest™ Enzymes – One Buffer System Compatible with All Downstream Applications

Fermentas FastDigest™ enzymes – a premium line of restriction enzymes for easy and robust digestion of DNA. All FastDigest™ enzymes work in the same reaction buffer and at the same temperature, therefore multiple digestions of DNA in any combination of FastDigest™ enzymes are simple!

Applications

- Fast clone analysis
- Fast preparation of DNA for cloning
- Digestion of PCR products
- Fast RFLP analysis
- Digestion of difficult-to-cleave DNA targets

YOUR BENEFITS

- *All enzymes work in same reaction conditions*
- *No more sequential digestions*
- *No more tedious buffer changes*
- *No more concerns about star activity*

Currently Fermentas provides 105 FastDigest™ enzymes and is continuously expanding the number. Visit www.fermentas.com/fastdigest for complete information and an up-to-date list of FastDigest™ enzymes.

One Universal Buffer

ONE reaction buffer • **ONE** reaction temperature • **ONE** µl of FastDigest™ enzyme • **ONE** µg of substrate DNA • **5 MINUTE** reaction

Multiple digestions of DNA in any combination of FastDigest™ enzymes are simple – all FastDigest™ enzymes work in the FastDigest™ Universal Buffer. No more concerns about buffer compatibility, no more sequential digestions or tedious buffer changes. Perform multiple digestion of DNA with FastDigest™ enzymes in 5 minutes!

The FastDigest™ Universal Buffer saves hours of time and effort!

Double Digestions with FastDigest™ Enzymes

	BamHI	BglII	EcoRI	HindIII	KpnI	NcoI	NdeI	NotI	PstI	PvuII	SacI	SalI	SmaI	XbaI	XhoI
BamHI															
BglII															
EcoRI															
HindIII															
KpnI															
NcoI															
NdeI															
NotI															
PstI															
PvuII															
SacI															
SalI															
SmaI															
XbaI															
XhoI															

– simply add two FastDigest™ enzymes to the reaction mixture and perform double digestion

Double Digestions with Restriction Enzymes from Another Vendor

	BamHI	BglII	EcoRI	HindIII	KpnI	NcoI	NdeI	NotI	PstI	PvuII	SacI	SalI	SmaI	XbaI	XhoI
BamHI															
BglII															
EcoRI	★														
HindIII	↓														
KpnI	↓		★												
NcoI															
NdeI															
NotI															
PstI															
PvuII	★		★												
SacI	↓		↓												
SalI				↓	↓						↓				
SmaI	↓	↓	↓		↓							↓			
XbaI	↓		★												
XhoI															

– sequential digestion

↓ – DNA must be purified prior to second digestion

– simply add two enzymes to the reaction mixture and perform double digestion

– necessary to use more units of one or both enzymes and to add BSA for complete DNA digestion

– necessary to use more units of one or both enzymes for complete DNA digestion

– necessary to add BSA to the reaction mixture

★ – star activity may occur in recommended conditions

Double Digestions with FastDigest™ Enzymes and Enzymes from Other Vendors

Fermentas FastDigest™ restriction enzymes		
1	ONE reaction mixture with: FastDigest™ BamHI, FastDigest™ SmaI	~2 min
2	Incubation	5 min
	TOTAL TIME	7 min

(Hands on time 2 min)

Restriction enzymes from other vendor		
1	Reaction mixture for BamHI	~2 min
2	Incubation	60 min
3	Purification of digested DNA	>15 min
7	Reaction mixture for SmaI	~2 min
8	Incubation	60 min
	TOTAL TIME	>139 min

(Hands on time ~19 min)

YOUR BENEFITS

- *Save hours and effort in multiple digestion with FastDigest™ enzymes*
- *No costly purification systems or precipitation reagents needed*
- *Compatible with common Fermentas DNA/RNA modifying enzymes*

Protocol for Double Digestion with FastDigest™ Enzymes

Digestion of single sample

1. Prepare the **reaction mixture** in a thin-wall tube at room temperature

Components	Volume
Water, nuclease free (#R0581)	variable
10X FastDigest™ buffer	2 µl
DNA (up to 1 µg)	variable
FastDigest™ enzyme 1	1 µl
FastDigest™ enzyme 2	1 µl
	Final volume 20 µl

2. Mix gently and spin down
3. Incubate* at 37°C for 5 minutes to digest DNA

Digestion of multiple samples

1. Pipette 2 µl (up to 1 µg) aliquots of DNA into thin-wall tubes at room temperature
2. Prepare the following **master mix** at room temperature:

Components	Volume
Water, nuclease free (#R0581)	(n+1) x 14 µl
10X FastDigest™ buffer	(n+1) x 2 µl
FastDigest™ enzyme 1	(n+1) x 1 µl
FastDigest™ enzyme 2	(n+1) x 1 µl

n –number of samples

Note If your substrate DNA is in a smaller volume, increase the volume of water, accordingly.

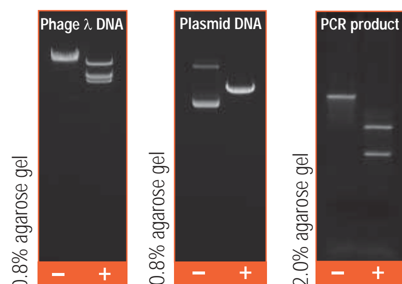
3. Mix gently and spin down
4. Incubate* at 37°C for 5 minutes to digest DNA

* Use a heat block or a water thermostat. Air thermostats are not recommended due to slow heat transfer to the reaction mixture.

FastDigest™ enzymes can be used for 5 minute digestion of any substrate DNA.
Only 1 µl of FastDigest™ enzyme is required to completely digest:

- up to 1 µg of lambda DNA
- up to 1 µg of plasmid DNA
- ~0.2 µg of PCR product

5 Minute Digestion of Phage λ DNA, Plasmid DNA and PCR Products with FastDigest™ BamHI



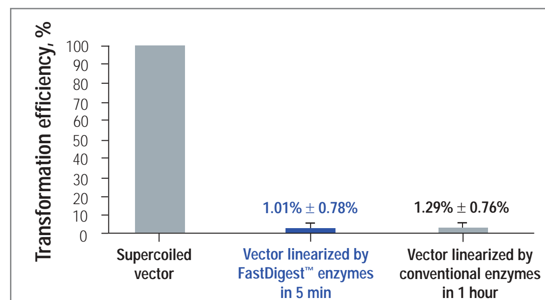
– – Control (without enzyme)
+ – Digested with 1 µl FastDigest™ BamHI (#ER0051)

Substrate DNA was incubated for 5 minutes with FastDigest™ BamHI (#ER0051) in 20 µl of reaction mixture.

Fast Preparation of Plasmid Vector for Cloning

Speed up your cloning experiments – digest plasmid vectors for cloning in 5 minutes with FastDigest™ enzymes!

Transformation of *E.coli* Cells with Vectors Linearized with FastDigest™ or Conventional Restriction Enzymes



The efficiency of plasmid vector cleavage was evaluated by transformation of *E.coli* cells with linearized vector (without gel-purification) after 5 minute digestion with 19 FastDigest™ enzymes and compared to efficiency of 1 hour conventional digestions with the corresponding 19 conventional restriction enzymes.

Protocol for Fast Vector Preparation with FastDigest™ Enzymes

1. Prepare the **reaction mixture** in a thin-wall tube at room temperature

Components	Volume
Water, nuclease free (#R0581)	variable
10X FastDigest™ buffer	2 µl
Plasmid DNA (up to 1 µg)*	variable
FastDigest™ enzyme	1 µl
	Final volume 20 µl

2. Mix gently and spin down
3. Incubate** at 37°C for 5 minutes to digest DNA

Scaling up DNA Digestion Reaction

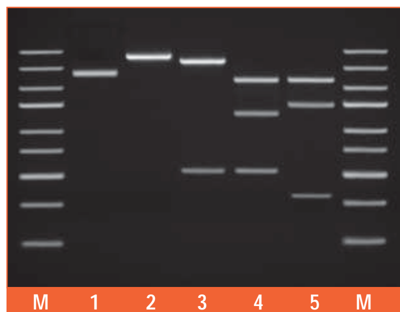
DNA	1 µg	2 µg	3 µg	4 µg	5 µg
FastDigest™ enzyme	1 µl	2 µl	3 µl	4 µl	5 µl
FastDigest™ buffer	2 µl	2 µl	3 µl	4 µl	5 µl
Total volume	20 µl	20 µl	30 µl	40 µl	50 µl

* For digestion of larger amounts of DNA, scale-up the reaction mixture (*see* table above).

** Use a heat block or a water thermostat. Air thermostats are not recommended due to slow heat transfer to the reaction mixture.

Save Time – Fast Clone Analysis with FastDigest™ Enzymes

Clone Analysis: 5 Minute DNA Digestion with FastDigest™ Enzymes



M – GeneRuler™ Express DNA Ladder (#SM1551)
1 – plasmid containing insert
2 – plasmid containing insert, digested with FastDigest™ NotI (#ER0594)
3 – plasmid containing insert, digested with FastDigest™ BamHI (#ER0054)
4 – plasmid containing insert, double digested with FastDigest™ NotI and FastDigest™ BamHI
5 – plasmid containing insert in the opposite orientation, double digested with FastDigest™ NotI and FastDigest™ BamHI

A recombinant pJET1 plasmid containing 953 bp insert, which carries one additional BamHI recognition site, was purified from recombinant *E.coli* culture using the GeneJET™ Plasmid Miniprep Kit (#K0501) and digested in 5 minutes with FastDigest™ restriction enzymes.

High quality plasmid DNA purified with the GeneJET™ Plasmid Miniprep Kit is ideal for fast digestion with FastDigest™ enzymes.

Protocol for Fast Digestion and Analysis of Plasmid DNA with FastDigest™ Enzymes

Digestion of single sample

1. Purify DNA from 1.5ml of *E.coli* culture using GeneJET™ Plasmid Miniprep Kit (#K0501)
2. Prepare the **reaction mixture** in a thin-wall tube at room temperature

Components	Volume
Water, nuclease free (#R0581)	15 µl
10X FastDigest™ buffer	2 µl
Plasmid DNA (up tp 1 µg)	2 µl
FastDigest™ enzyme	1 µl

3. Mix gently and spin down
4. Incubate* at 37°C for 5 minutes to digest DNA
5. Add 4 µl of 6X loading dye solution into each tube and mix
6. Load on a 0.8-1% agarose gel and run electrophoresis** for 10-20 min

Digestion of multiple samples

1. Purify DNA from 1.5ml of *E.coli* culture using GeneJET™ Plasmid Miniprep Kit (#K0501)
2. Pipette 2 µl (up to 1 µg) aliquots of miniprep DNA into thin-wall tubes
3. Prepare the following **master mix** at room temperature:

Components	Volume
Water, nuclease free (#R0581)	(n+1) x 15 µl
10X FastDigest™ buffer	(n+1) x 2 µl
FastDigest™ enzyme	(n+1) x 1 µl

n –number of samples

4. Add 18 µl of the master mix into each tube with plasmid DNA
5. Mix gently and spin down
6. Incubate* at 37°C for 5 minutes to digest DNA
7. Add 4 µl of 6X loading dye solution into each tube and mix
8. Load on a 0.8-1% agarose gel and run electrophoresis** for 10-20 min

* Use a heat block or a water thermostat. Air thermostats are not recommended due to slow heat transfer to the reaction mixture.

** Use Fermentas DNA Ladders for fast electrophoresis: ZipRuler™ DNA Ladder Set (#SM1373), GeneRuler™ Express DNA Ladder (#SM1551) or FastRuler™ DNA Ladder, Middle Range (#SM1113).

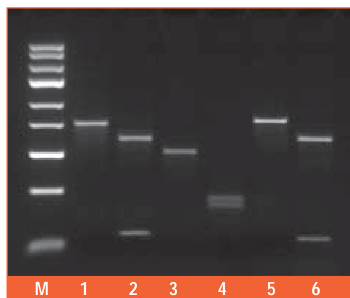
Fast Digestion of PCR Products without Compromise!

Digestion of PCR-amplified DNA by restriction enzymes is often inefficient and is dependent on many complex factors. For achieving complete digestion of amplified DNA, careful purification of the PCR product is often required.

Fermentas FastDigest™ enzymes are ideal for complete digestion of most non-purified PCR products in just 5 minutes. For "difficult substrates" 1 hour digestion will work with no star activity. PCR products digested with FastDigest™ enzymes maintain the integrity of DNA ends and can be effectively used for cloning or other applications.

Just mix an aliquot of PCR mixture with FastDigest™ buffer and water, add the FastDigest™ enzyme and incubate. No additional steps are required!

Digestion of Unpurified PCR Products in 5 Minutes with FastDigest™ Enzymes



M – GeneRuler™ Express DNA Ladder (#SM1551)
1 – PCR product (undigested)
2 – PCR product digested with FastDigest™ NcoI (#ER0574)
3 – PCR product (undigested)
4 – PCR product digested with FastDigest™ RsaI (#ER1124)
5 – PCR product (undigested)
6 – PCR product digested with FastDigest™ MspI (#ER0544)

~0.2 µg of unpurified PCR product was digested in 5 minutes with FastDigest™ enzymes in a 20 µl reaction mixture at 37°C temperature.

Protocol for Fast Digestion of PCR Products with FastDigest™ Enzymes

Digestion of single sample

1. Perform PCR*
2. Prepare the **reaction mixture** in a thin-wall tube at room temperature

Components	Volume
Water, nuclease free (#R0581)	17 µl
10X FastDigest™ buffer	2 µl
PCR product (~0.2 µg)	10 µl
FastDigest™ enzyme	1 µl

3. Mix gently and spin down
4. Incubate** at 37°C for 5 minutes to digest DNA

Digestion of multiple samples

1. Perform PCR*
2. Pipette 10 µl (~0.2 µg) of each PCR product into thin-wall tubes
3. Prepare the following **master mix** at room temperature:

Components	Volume
Water, nuclease free (#R0581)	(n+1) x 17 µl
10X FastDigest™ buffer	(n+1) x 2 µl
FastDigest™ enzyme	(n+1) x 1 µl

n –number of samples

4. Add 20 µl of the master mix into each tube, mix gently and spin down
5. Incubate** at 37°C for 5 minutes to digest DNA

Note

- PCR additives such as DMSO or glycerol may affect the cleavage efficiency of FastDigest™ enzymes or cause star activity.
- In some cases, the context of the target sequence may affect cleavage of PCR products, resulting in incomplete cleavage after 5 minutes. In these cases, a 30 min incubation with the FastDigest™ enzyme is recommended.
- When introducing restriction enzyme sites into primers for subsequent digestion and cloning of a PCR product, refer to the Table "Cleavage efficiency close to the termini of PCR fragments" (www.fermentas.com) to define the number of extra bases required for efficient cleavage.
- For double digestions of PCR products reduce the amount of water by 1 µl and add 1 µl of the second FastDigest™ enzyme to the reaction mixture or master mix.
- For cloning applications, purification of PCR products prior to digestion is highly recommended to remove the active thermophilic DNA polymerase still present in the PCR mixture. DNA polymerases may alter the ends of the cleaved DNA and reduce the ligation yield.

* Perform PCR with Fermentas *Taq* DNA Polymerase (#EP0401, #EP0281, #EP0071), Hot Start *Taq* DNA Polymerase (#EP0601) or TrueStart™ *Taq* DNA Polymerase (#EP0611), *Pfu* DNA Polymerase (#EP0501, #EP0571), High Fidelity PCR Enzyme Mix (#K0191), Long PCR Enzyme Mix (#K0181) in the supplied buffers or use 2X PCR Master Mix (#K0171) or GeneJET™ Fast PCR Master Mix (2X) (#K0211).

** Use a heat block or a water thermostat. Air thermostats are not recommended due to slow heat transfer to the reaction mixture.

Fermentas – the Leader in Restriction Enzyme Quality

Fermentas restriction enzymes are PureExtreme® Quality – the highest quality and performance enzymes in the field.

What does PureExtreme® Quality Mean for You?

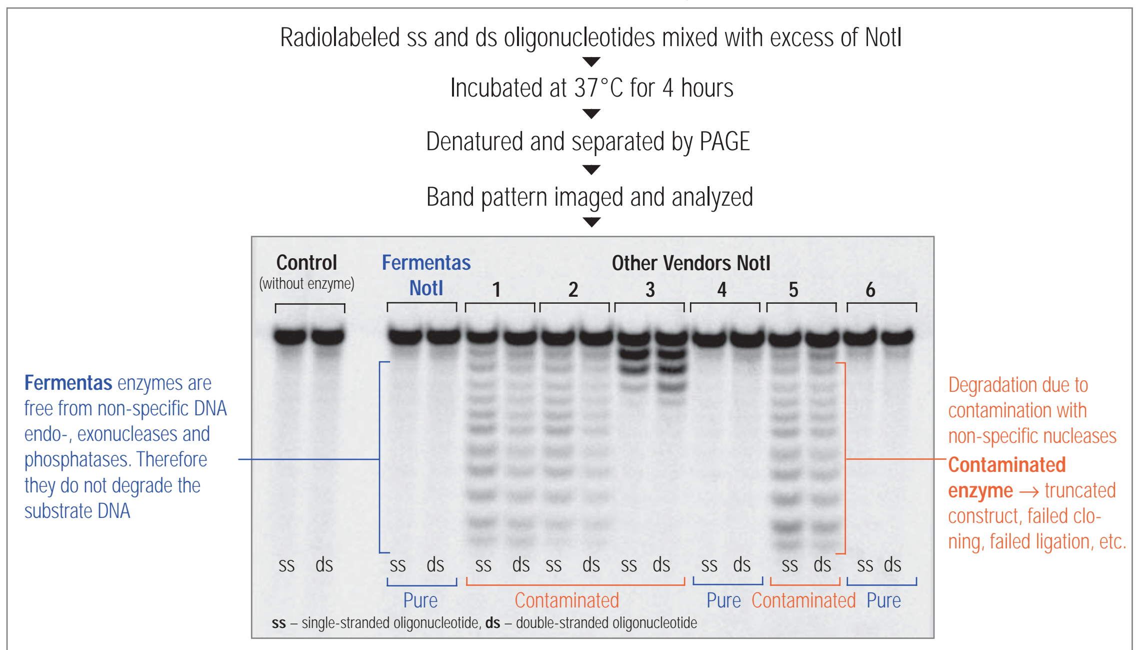
- Restriction enzymes free of contaminating activities (DNases, phosphatases)
- Stringent quality control with the most advanced tests
- ISO9001 & ISO14001 is your assurance of consistency and lot-to-lot reproducibility
- The performance you need for your most demanding experiments
- Quantitative digests, due to guaranteed enzyme concentrations
- Successful clonings, due to integrity of DNA ends
- Less minipreps necessary to identify positive clones

Fermentas restriction enzymes are assayed with the most stringent quality control tests in the field. We have developed the unique test of functional purity – the Labeled Oligonucleotide (LO) test. The LO test is the most sensitive assay for detection of trace amounts of non-specific DNA endonucleases, exonucleases and phosphatases in enzyme preparations (*see* Figure "The Labeled Oligonucleotide (LO) Test").

Fermentas restriction enzymes are also assayed in conventional quality control tests such as: non-specific nuclease and cross-contamination assay, ligation and recleavage assay and Blue/White cloning assay.
Even in conventional assays, we set higher standards compared to our competitors:

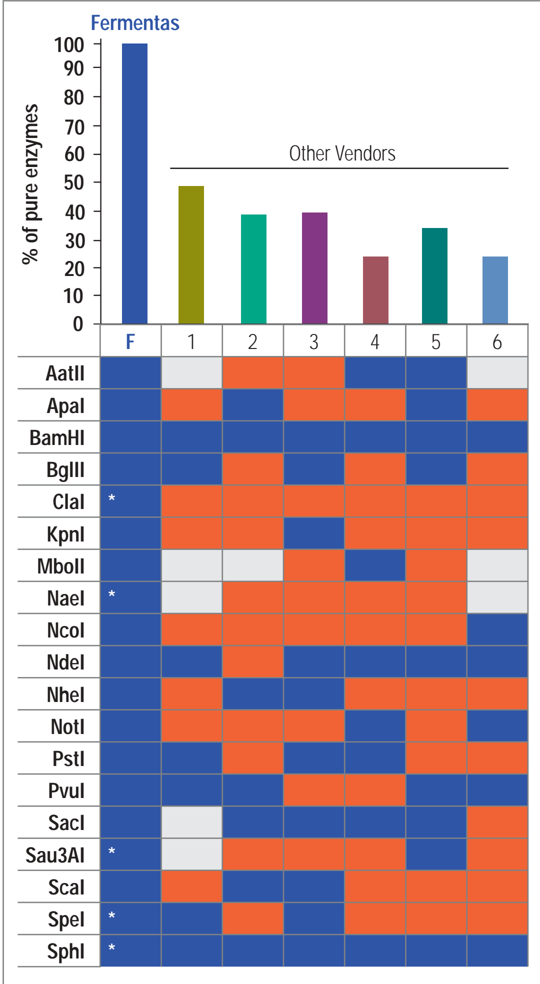
- We use a much higher functional excess of enzyme to detect contaminants
- We perform the Blue/White cloning with multiple substrates, allowing us to assay for the integrity of 5′-, 3′- and blunt ends after DNA vector digestion
- We monitor all enzyme lots for adherence to control specifications right to the expiry date

The Labeled Oligonucleotide (LO) Test – the Most Sensitive Assay in the Field
Evaluation of NotI Purity



We have tested restriction enzymes from different vendors and found that many of them are not pure enough to pass our standard quality control assay – the Labeled Oligonucleotide test (*see* table below).

ALL Fermentas Restriction Enzymes Pass the LO Test



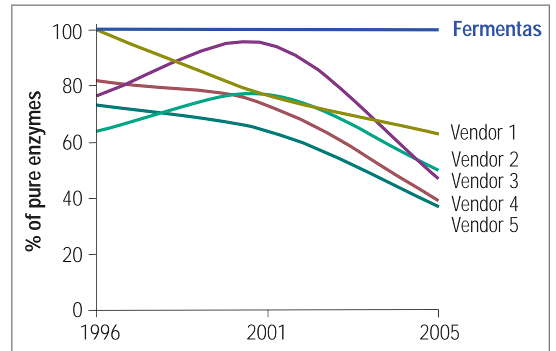
* corresponding isoschizomers of the prototypes were used

– pure enzyme

– contaminated enzyme

– not available

Consistency of the Fermentas PureExtreme® Quality Restriction Enzymes Over the Years



Purity of restriction enzymes from different vendors was evaluated by the LO test.

YOUR BENEFITS

- ***PureExtreme® Quality enzymes***
- ***Lot-to-lot consistency***
- ***Reliable supplier***

Lot-to-Lot Reproducibility Leads to Consistent Results

At Fermentas the PureExtreme® Quality of restriction enzymes is our highest priority. Restriction enzymes are produced under ISO9001 quality and ISO14001 environmental management systems that are our guarantee of consistency and lot-to-lot reproducibility.

Fermentas PureExtreme® Product

ISO9001 and ISO14001

- **Manufacturing**
- **Quality Control**
  - Stringent quality control tests
    - Labeled Oligonucleotide (LO) test
    - Non-specific nuclease and cross-contamination assay
    - Ligation and recleavage assay
    - Blue/White (B/W) cloning assay
- **Packaging**
  - Color-coded vials
  - Expiry date on every vial
  - Uniform concentation (10 u/µl)
  - High concentration (50 u/µl) for popular enzymes
  - Big packs aliquoted in separate vials
  - Low concentrations (1-5 u/µl) for enzymes with higher price/unit
- **Storage**
  - Monitoring of enzyme quality until the expiry date
- **Delivery**
- **Customer Service**
  - Customer feedback analysis

YOUR BENEFITS

Lot-to-lot consistency | Free of contaminating activities | User-friendly packaging | Guaranteed performance | Fast delivery | Innovative products e.g. FastDigest™ enzymes

No Cloning Background with Fermentas Enzymes

Molecular cloning is one of the main applications of restriction enzymes. Cloning experiments are extremely sensitive to contaminating activities that may be present in enzyme preparations, such as non-specific nucleases and phosphatases.

- Trace contamination with 5′- and 3′-exonucleases can cause DNA inserts and cloning vectors to degrade. This is often the cause of cloning background, false positive colonies and other cloning artifacts
- Non-specific DNA endonucleases may cause cloning of incomplete inserts or damaged vectors
- Phosphatase contamination may impair ligation and lead to reduced number of clones

Every lot of Fermentas PureExtreme® restriction enzymes is free of non-specific DNA endonucleases, exonucleases and phosphatases (*see* page 10 for a description of the LO test). This ensures that the integrity of DNA ends is maintained after digestion and guarantees the best performance in molecular cloning.

Evaluation of Functional Enzyme Purity

Functional testing of the integrity of DNA ends was tested using positive selection cloning vector (pJET1) containing a toxic gene. The plasmid was digested within the toxic gene using NcoI from different vendors, recircularized by ligation and transformed into *E.coli* cells.

NO background colonies

Pure enzyme
Fermentas' NcoI

False positive colonies

Contaminated enzyme
Vendor's 1 NcoI

NO colonies

NO enzyme
Control

NO background colonies – DNA was not damaged during restriction digestion (no cross contaminating activities).

False positive colonies – toxic gene was inactivated due to DNA damage by cross contaminating activities of the vendor's restriction enzyme.

Control pJET1 (not digested with the enzyme) incubated with buffers and ligase.

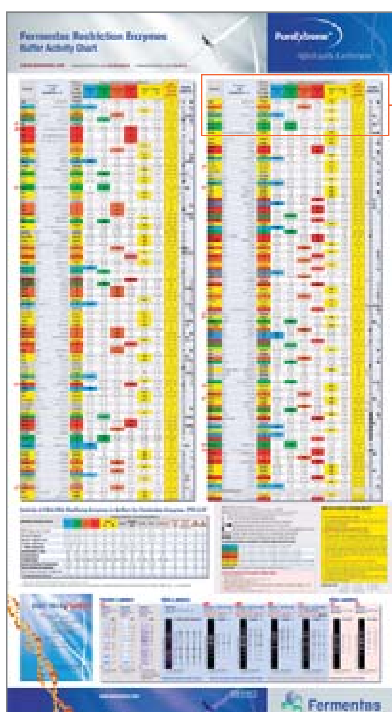
Vendor	Number of background colonies
Fermentas	0
Vendor 1	134
Vendor 2	4
Vendor 3	43
Vendor 4	36
Vendor 5	28

❶ Find the enzyme you need

Use the **REsearch™ engine** at www.fermentas.com/research to search for restriction enzymes using either the enzyme name (or part of it), or the recognition sequence. You will be provided with detailed information about the properties of each Fermentas restriction enzyme

❷ Perform digestion of DNA

- Use FastDigest™ enzymes for fast and convenient DNA digestion (*see* page 4)
- Use conventional restriction enzymes for routine digestions. You can find the reaction conditions best matching to your experiment design in poster "Fermentas Restriction Enzymes. Buffer Activity Chart"



Enzyme	Prototype and specificity 5'→3'	Recom-mended buffer for 100% activity	Enzyme activity in Fermentas buffers, %: B (blue) 1X	G (green) 1X	O (orange) 1X	R (red) 1X	Tango™ (yellow) 1X	Tango™ (yellow) 2X	Tango™ buffer for double digestion	Enzyme properties
FaqI	FinI GGGAC(10/14)↓	Tango™ (+SAM)	20-50 (+SAM)	20-50 (+SAM)	0-20 (+SAM)	0-20 (+SAM)	100 (+SAM)	20-50 (+SAM)	1X or 2X (+SAM)	
FspAI	FspAI RTGC↓GCAY	O	0-20	0-20	100	50-100	0-20	50-100	2X	
FspBI	MaeI C↓TAG	Tango™	50-100	20-50	0-20	0-20	100	0-20	1X	
GsuI	GsuI CTGGAG(16/14)↓	B	100	50-100	20-50	20-50	100	50-100	1X or 2X	
HhaI	HhaI GCG↓C	Tango™	50-100	50-100	20-50	20-50	100	20-50	1X or 2X	
Hin1I	AcyI GR↓CGYC	G	20-50	100	20-50	20-50	20-50	20-50	1X or 2X	
Hin1II	NlaIII CATG↓	G	50-100	100	20-50	50-100	50-100	50-100	1X or 2X	
Hin4I	Hin4I ↓(8/13-14)GAY(N)VTC(13-14/8)↓	Tango™ (+SAM)	20-50 (+SAM)	20-50 (+SAM)	0-20 (+SAM)	0-20 (+SAM)	100 (+SAM)	0-20 (+SAM)	1X (+SAM)	

Color-coded enzyme tube corresponds to the recommended buffer

Color-coded recommended buffer (100% activity of enzyme in the recommended buffer)

Relative activity, compared to the activity in the recommended buffer

Tango™ buffer concentration for double digestion

Ask your distributor for a free copy of the poster or download pdf file from the www.fermentas.com.

Protocol for Digestion of DNA with Conventional Restriction Enzymes

❶ Add components in the following order:

Components	Amount
Water, nuclease free (#R0581)	16 µl
10X recommended buffer	2 µl
Substrate DNA (~1 µg)	1 µl
Restriction enzyme	0.5-1 µl

❷ Mix gently and spin down

❸ Incubate at the optimum temperature for 1-16 hours

The digestion reaction may be scaled up and down

Note

- Some enzymes require additional components to obtain the stated activity. In these cases, add the required additive and adjust the volume of water appropriately.

Protocol for Digestion of PCR Products with Conventional Restriction Enzymes

All Fermentas restriction enzymes have been certified for compatibility with reagents and buffers used in PCR – the restriction enzyme can be added directly to the reaction mixture after amplification.

1. Add components in the following order:

Components	Amount
PCR reaction mixture (~0.1-0.5 µg of DNA)	10 µl
Water, nuclease free (#R0581)	16-17 µl
10X recommended buffer	2 µl
Restriction enzyme (10-20 u)	1-2 µl

2. Mix gently and spin down
3. Incubate at the optimum temperature for 1-16 hours

Note

- If the diluted PCR products are incompletely digested, purify the PCR products with the DNA Extraction Kit (#K0513), then digest the purified DNA.
- For cloning applications, purification of PCR products prior to digestion is highly recommended to remove the active thermophilic DNA polymerase still present in the PCR mixture. DNA polymerases may alter the ends of the cleaved DNA and reduce the ligation yield.
- If a restriction enzyme requires special additives (e.g., SAM), reduce the amount of water appropriately.
- PCR additives such as DMSO or glycerol may affect the cleavage efficiency of restriction enzymes or cause star activity.
- When introducing restriction enzyme sites into primers for subsequent digestion and cloning of a PCR product, refer to the Table "Cleavage efficiency close to the termini of PCR fragments" (www.fermentas.com) to define the number of extra bases required for efficient cleavage.

❸ Perform double digestion of DNA

- Use FastDigest™ enzymes for convenient double digestions. All FastDigest™ enzymes are 100% active in the same universal buffer therefore double digestions of DNA in any combination of FastDigest™ enzymes are simple (*see* page 5)
- For double digestion with conventional restriction enzymes:
  - use the **DoubleDigest™ engine** at www.fermentas.com/doubledigest to automatically find the conditions for double digestion. Just select two restriction enzymes, submit the query and follow the recommendations
  - use the restriction enzyme poster "Fermentas Restriction Enzymes. Buffer Activity Chart"

❹ Perform downstream applications directly

All common DNA/RNA modifying enzymes are active in Fermentas restriction enzyme buffers, so there is no need to change the reaction buffer when performing downstream applications

- Refer to www.fermentas.com/techinfo/modifyingenzymes/modenzactivity.htm to choose the best conditions for your downstream applications
- Use the restriction enzyme poster "Fermentas Restriction Enzymes. Buffer Activity Chart" table "Activity of DNA/RNA Modifying Enzymes in Buffers for Restriction Enzymes, PCR & RT"

❺ Analyze and plan experiments

- COMING SOON **REviewer™** – a multifunctional tool at www.fermentas.com/reviewer for DNA sequence analysis and visualization:
  - plasmid drawing
  - identification of recognition sequences
  - virtual DNA digestion
  - ability to save your sequences for later use
  - easy conversion of your sequences into the most popular formats
  - primer selection and calculation of oligonucleotide properties (such as Tm, dG, etc)
  - biochemical calculations: molecular weight, conversions between molar quantities and mass quantities, etc.

TECHNICAL INFORMATION

Technical Information name of chart/article presented in Fermentas literature	Content	Where to find? web	 catalog	 COA*	 poster
HOW TO FIND THE ENZYME YOU NEED					
Alphabetic list of commercially available restriction enzymes	Fermentas enzymes listed according to commonly used prototype restriction enzyme names	✓	✓		
Recognition specificities	Enzyme listed according to recognition sequence	✓	✓		
Commercial restriction enzymes sorted by type of DNA ends generated	Enzymes listed according to compatible ends for ligation	✓	✓		
Newly generated recognition sequences	Predicts generation of new recognition sequences after: • Removal of 3′-overhang and self-ligation • Fill-in of a 5′-overhang and self-ligation • Ligation of blunt ends generated by two enzymes • Ligation of protruding compatible DNA ends	✓	✓		
Site preferences by restriction enzymes	Suggestions for efficient digestion of difficult-to cleave recognition sequences	✓	✓		
Digestion of methylated DNA	List of isoschizomers with differing sensitivities to the target methylation	✓	✓	✓	
	Effects of methylation (Dam, Dcm, CpG, EcoKI and EcoBI) on DNA digestion by restriction enzymes	✓	✓	✓	✓
Number of recognition sites in DNA molecules	Number of recognition sites in most popular phage and plasmid DNAs	✓	✓	✓	
Cleavage of restriction targets located within the pUC19 multiple cloning site (MCS)	Efficient double digestion within pUC19 MCS; enzymes for the first and for the second digestion are recommended	✓	✓		
HOW TO PERFORM DNA DIGESTION REACTIONS					
Reaction buffers for restriction enzymes	Composition of the Fermentas Five Buffers and unique buffers	✓	✓	✓	✓
Protocol for DNA digestion	Protocol for digestion with conventional enzymes	✓	✓	✓	
Fast digestion and analysis	Protocol for digestion with FastDigest™ enzymes	✓	✓	✓	
Dilution of restriction enzyme	Composition of buffer for dilution of restriction enzymes	✓	✓	✓	
Activity of mesophilic and thermophilic enzymes at 37°C	Optimal temperature of mesophilic and thermophilic enzymes and their activity at 37°C	✓	✓	✓	✓
Star activity	Suggestions on how to avoid star activity	✓	✓	✓	✓
Conditions to thermal inactivation	Susceptibility of restriction enzymes to thermal inactivation and inactivation temperatures	✓	✓	✓	✓
Stability during prolonged incubations	Possibility to save enzyme by performing overnight digestion	✓	✓	✓	
Double digestions using universal Tango™ buffer	Tango™ buffer concentration (1X or 2X) for double digestions	✓	✓	✓	✓
Double digestion with FastDigest™ enzymes	Protocol	✓	✓	✓	
DNA purification after enzymatic reaction by phenol/chlorophorm extraction and alcohol precipitation	Protocol	✓	✓	✓	
Buffer conversion guide	Compatibility of Fermentas restriction enzymes buffers with buffers from other vendors	LabAid™ "Buffer Conversion Guide"			
Considerations for partial digestion of DNA	Recommendations on how to achieve incomplete cleavage of DNA	✓	✓		
Guide for successful digestions	Troubleshooting of digestion reaction	✓	✓		
HOW TO DIGEST PCR PRODUCTS					
Cleavage efficiency close to the termini of PCR fragments	Lists how many extra nucleotides are required when designing PCR primers with restriction sites	✓	✓		
Digestion of PCR products with conventional restriction enzymes	Protocol	✓	✓	✓	
Digestion of PCR products with FastDigest™ enzymes	Protocol	✓	✓	✓	
Guide for successful digestions	Troubleshooting of digestion reaction	✓	✓		
LOT-SPECIFIC INFORMATION					
Activity assay	Unit definition conditions of restriction enzymes	✓	✓	✓	
Quality control	Quality control assay data	✓	✓	✓	
Storage and shipping	Temperature requirements for storage and shipping	✓	✓	✓	
GENERAL INFORMATION					
Activity of DNA/RNA modifying enzymes in buffers for restriction enzymes	Compatibility of restriction enzyme buffers with downstream applications	✓	✓		✓
Guide for successful digestions	Troubleshooting of digestion reaction	✓	✓		

* Certificate of Analysis

DoubleDigest, **FastDigest**, **FastRuler**, **GeneJET**, **GeneRuler**, **REsearch**, **REviewer, Tango** and **ZipRuler** are Fermentas trademarks. **PureExtreme** is registered Fermentas trademark.

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