



High Fidelity (HF) Restriction Enzymes

New England Biolabs provides customers with high quality tools for a wide range of molecular biology applications. As part of our ongoing commitment to the study and improvement of restriction enzymes, we are pleased to introduce a line of restriction enzymes that have been engineered for maximum performance, convenience and flexibility.

These engineered enzymes have the same specificity as their established counterparts. However, certain properties have been altered, including buffer requirements and enzyme fidelity. These modifications provide more flexibility in setting up their restriction enzyme digests. The overall goal of engineering restriction enzymes is to provide improved enzymes that will allow more flexibility with respect to reaction volume, incubation time and buffer compatibility. Each of these enzymes has been purified to the same high standards as our other restriction enzymes.

The introductory selection of engineered restriction enzymes offers the benefit of reduced star activity. Star activity, or relaxed specificity, is an intrinsic property of restriction enzymes. Most restriction enzymes will not exhibit star activity when used under recommended reaction conditions. However, for enzymes that have reported star activity, extra caution must be taken to set up reactions carefully under the recommended conditions to avoid unwanted cleavage. Different techniques such as cloning, genotyping, mutational analysis, mapping, probe preparation, sequencing and methylation detection employ a wide range of reaction conditions and require the use of enzymes under suboptimal conditions. These new products with reduced star activity will offer increased flexibility to reaction setup, maximizing results under a wider range of conditions.

In addition to reduced star activity, all of these HF restriction enzymes work optimally in NEBuffer 4, which has the highest level of enzyme compatibility (currently, 162 NEB enzymes are recommended for use in NEBuffer 4), simplifying double digest reactions. NEB's HF enzymes are also Time-Saver™ qualified, and digest substrate DNA in five minutes (for more information on Time-Saver, see www.neb.com).

In order to distinguish these engineered enzymes, the letters -HF™ have been added to the restriction enzyme name. An icon designating that the enzyme has been engineered (e) appears with each product entry, on the datacard and on the website. In addition, icons for the enhanced properties that these new enzymes will possess are included. These enzymes are packaged with a purple cap to distinguish them from our existing enzymes.

Visit our website, www.neb.com to learn more about the latest innovation in restriction enzyme technology from New England Biolabs.

CLONING & MAPPING

DNA AMPLIFICATION & PCR

RNA ANALYSIS

PROTEIN EXPRESSION & ANALYSIS

GENE EXPRESSION & CELLULAR ANALYSIS

High fidelity enzymes now available:

- BamHI-HF™
- EagI-HF™
- EcoRI-HF™
- EcoRV-HF™
- MfeI-HF™
- NcoI-HF™
- NheI-HF™
- NotI-HF™
- PvuII-HF™
- SacI-HF™
- SalI-HF™
- SbfI-HF™
- ScaI-HF™
- SphI-HF™
- SspI-HF™

HF™-Factor: Reduced star activity of HF™ Enzymes in relation to their wild type counterparts


PRODUCT NAME	PRODUCT NUMBER	BUFFER†	HF™-FACTOR: star activity reduced by*
BamHI-HF™	#R3136S	4	125x
EagI-HF™	#R3505S	4	2x
EcoRI-HF™	#R3101S	4	64x
EcoRV-HF™	#R3195S	4	64x
MfeI-HF™	#R3589S	4	15x
NcoI-HF™	#R3193S	4	133x
NheI-HF™	#R3131S	4 + BSA	266x


PRODUCT NAME	PRODUCT NUMBER	BUFFER†	HF™-FACTOR: star activity reduced by*
NotI-HF™	#R3189S	4 + BSA	16x
PvuII-HF™	#R3151S	4	31x
SacI-HF™	#R3156S	4 + BSA	33x
SalI-HF™	#R3138S	4	500x
SbfI-HF™	#R3642S	4	31x
ScaI-HF™	#R3122S	4	62x
SphI-HF™	#R3182S	4	62x

† Wild type enzymes were tested in supplied buffer for comparisons.

* For experimental details see Wei, H. et al (2008) *Nucleic Acids Reseach* 36, e50.

RESTRICTION ENZYMES


BamHI-HF™ 
 5'... G[▼]GATCC...3'
 3'... CCTAG[▲]G...5'
 # R3136S 5,000 units


EagI-HF™ 
 5'... C[▼]GGCCG...3'
 3'... GCCGG[▲]C...5'
 # R3505S 20,000 units/ml

EcoRI-HF™ 
 5'... G[▼]AATTC...3'
 3'... CTTAA[▲]G...5'
 # R3101S 10,000 units

EcoRV-HF™ 
 5'... GATATC...3'
 3'... CTA[▲]TAG...5'
 # R3195S 4,000 units

MfeI-HF™ 
 5'... C[▼]AATTG...3'
 3'... GTTAA[▲]C...5'
 # R3589S 500 units/ml

NcoI-HF™ 
 5'... C[▼]CATGG...3'
 3'... GGTAC[▲]C...5'
 # R3193S 1,000 units


NheI-HF™ 
 5'... G[▼]CTAGC...3'
 3'... CGATC[▲]G...5'
 # R3131S 1,000 units

NotI-HF™ 
 5'... G[▼]CGGCCGC...3'
 3'... CGCCG[▲]GCG...5'
 # R3189S 500 units

PvuII-HF™ 
 5'... CAG[▼]CTG...3'
 3'... GTC[▲]GAC...5'
 # R3151S 5,000 units


SacI-HF™ 
 5'... GAG[▼]CTC...3'
 3'... C[▲]TCGAG...5'
 # R3156S 2,000 units

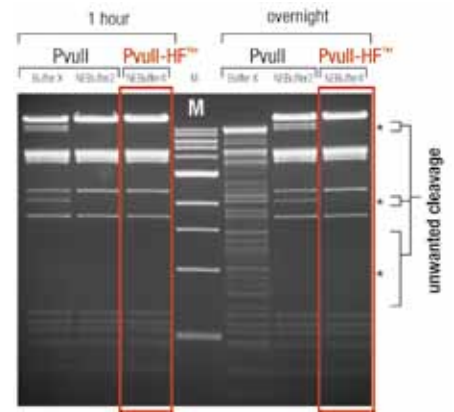
SalI-HF™ 
 5'... G[▼]TCGAC...3'
 3'... CAG[▲]CTG...5'
 # R3138S 2,000 units

SbfI-HF™ 
 5'... CCTGC[▼]AGG...3'
 3'... GG[▲]ACGTCC...5'
 # R3642S 500 units












ScaI-HF™ 
 5'... AGT[▼]ACT...3'
 3'... TCA[▲]TGA...5'
 # R3122S 1,000 units

SphI-HF™ 
 5'... G[▼]CATGC...3'
 3'... C[▲]GTAG...5'
 # R3182S 500 units

SspI-HF™ 
 5'... AATA[▼]AAT...3'
 3'... TAA[▲]TAA...5'
 # R3132S 1,000 units



PvuII can exhibit star activity when used under suboptimal reaction conditions. This activity is significantly reduced with PvuII-HF, even under extended PvuII shows 100% activity in all four NEBuffers, but exhibits intrinsic star activity in buffers other than the recommended NEBuffer 2 or under extended incubation times (i.e. overnight). Star activity is not observed with PvuII-HF™, even in overnight digests! (20 µl reactions were set up using 2 µl of enzyme and incubated for the indicated time at 37°C. Marker M is the 1 kb DNA Ladder (NEB #N3232).

 Cloned at NEBiolabs	 37°C Incubation Temperature
 Recombinant Enzyme	 dam Methylation Sensitivity
 Time-Saver Qualified	 Yes Heat Inactivation
 Optimum Buffer	 Blue/White Certified
 Requires BSA	
NEW ICONS:	
 Indicates that the enzyme has been engineered	 Indicates that the enzyme has reduced star activity

References

1. New England Biolabs, unpublished observations.
2. Nasri M. and Thomas D. (1987) *Nucleic Acids Res.*, 15, 7677.
3. Malyguine E. and Vannier P. and Yot. (1980) *Gene*, 8, 163.
4. Verdone L. et al. (1996) *Mol. and Cell. Biol.*, 16, 1978-1988.