

## HIGHLIGHTS OF GENEI RESTRICTION ENZYMES

Bangalore Genei restriction enzymes are supplied in convenient pack sizes and suitable concentrations. We are supplying commonly used enzymes in high concentration to aid genomic DNA digestions. Each lot of enzyme is rigorously checked for its integrity and functional purity during and after the process. All batches of restriction enzymes are assayed for their activity once every two months. Stability checks have confirmed that most of the enzymes are stable at -20° C for more than one year. Concentrated enzymes stay active longer than dilute enzymes.

We have divided the restriction enzymes based on the assay conditions into five groups for the convenience of the user. The enzymes and the buffer vials are colour coded. 10X assay buffer and 100X nuclease free BSA (wherever needed) is supplied free of cost with the enzyme. Bangalore Genei also caters to your additional requirement of reaction buffers. For reaction buffer set refer index. Unique buffer is supplied for the enzyme which performs suboptimally with the existing five assay buffers. The details of incubation buffer system is given in the catalogue.

### QUALITY CHECKS

#### Unit Definition:

The catalytic activity of the restriction enzymes available from us is based on the determination of the minimum amount of enzyme required for the generation of the enzyme-specific final fragment pattern of lambda DNA in most cases.

**One unit is defined as the amount of enzyme required to produce a complete digest of 1 µg of lambda DNA in a reaction volume of 50 µl in 60 minutes under optimal conditions of salt, pH and temperature.**

In some cases the determination of the unit is based on digestion of  $\lambda$  *dam*- DNA, pBR 322 DNA, Ad2 DNA,  $\lambda$  / *EcoR* I digest or  $\lambda$  / *Hind* III digest.

The appropriate incubation temperature is generally 37° C. There are exceptions like *Sma* I is incubated at 25° C, *Bcl* I at 50° C, *BstE* II at 60° C and *Taq* I at 65° C.

#### Overnight Non-specific Nuclease Assay:

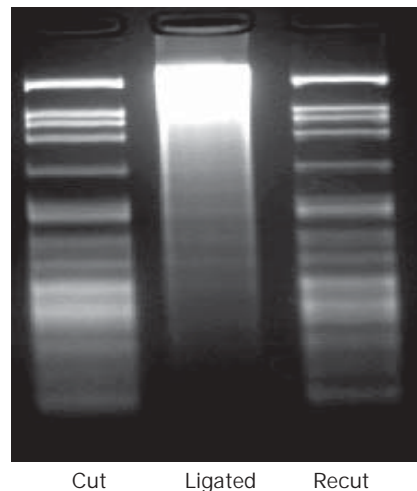
Every preparation of restriction enzyme is tested for non-specific nucleases. 10-50 units of enzyme is incubated with 1 µg of substrate DNA at the recommended assay conditions in 50 µl reaction volume for 16 - 20 hours. A sharp unaltered banding pattern is an indication of the purity of enzyme free from detectable non-specific nucleases. The highest number of units producing sharp unaltered pattern is reported on the certificate of analysis supplied with each enzyme.

#### Non-Specific Endonuclease assay:

The test is done for enzymes that do not have any site in supercoiled plasmid substrate. These enzymes are incubated with 1 µg of supercoiled (RFI form) DNA in 0.05 ml reaction volume for 4 hours at the recommended assay conditions. A single non-specific nick in the supercoiled form produces nicked (RF-II) form. The two forms can be distinguished on an agarose gel and the percentage of conversion estimated.

#### Ligation/Recut Assay:

The DNA fragments produced by a 3 to 10 fold excess of restriction enzyme digestion are ligated. Following the ligation, the DNA fragments are recut with the same restriction enzyme. An unaltered banding pattern after cleavage indicates intact 5' and 3' termini as well as the absence of contaminating nucleases. The estimated level of ligation and recleavage must exceed 70-90%.



Ligation Recut assay of Lambda/*Hae* III

**Blue/White Standard Assay:**

This is a very sensitive assay for the restriction enzymes used in cloning applications. This assay allows the detection of very low levels of nuclease contamination present in restriction enzymes. This assay is performed for those enzymes that have site present in the multiple cloning region that is in turn present within Lac Z $\alpha$  gene of the plasmid.

The assay is performed by cleaving the plasmid with 3-10 fold excess of enzyme. The cleaved DNA is ligated and used to transform competent DH5 $\alpha$  *E. coli* cells. The transformed cells are grown on a selective medium to ensure that all the colonies that grow arise from an *E. coli* transformed with the ligated vector. These colonies are tested for the integrity of the polylinker site by checking for the presence of the functional  $\beta$ -galactosidase locus. Only those colonies that turn blue on addition of X-gal and IPTG have  $\beta$ -galactosidase locus intact that in turn indicates that the polylinker site (that lies within this structural gene) was unchanged by cut and religation. The permitted percentage of white colonies is upto 3% for sticky end generating enzymes and upto 10% for blunt end generating enzymes.

**Star activity:** Bangalore Genei recommends very careful use of a few enzymes which are very sensitive to slightly altered assay conditions. The enzymes which exhibit star activity when high units/ $\mu$ g of DNA and/or incubated for long hours are *Bam*H I, *Eco*R I, *Kpn* I, *Nco* I, *Hinf* I, *Pvu* II, *Sau*3A I, *Ssp* I, *Sal* I, *Nhe* I and *Kpn* I.

## Tips to use Restriction Enzymes

- All restriction enzymes and 10X assay buffers should be stored at -20°C. Thaw the assay buffers completely before use.
- Restriction enzymes should be kept on ice when they are not in the freezer.
- The enzyme should always be the last component added to the reaction mixture.
- The substrate DNA should be free of contaminations such as phenol, chloroform, alcohol, EDTA, detergents or excessive salts all of which can interfere with restriction enzyme activity.
- Nature of DNA and DNA Methylation is also an important element of a restriction digestion so it is advisable to check the amount of enzyme needed to cleave the substrate prior to the actual experiment.
- The restriction enzyme: DNA: reaction volume ratio according to the unit definition is 1 U: 1  $\mu$ g: 50  $\mu$ l and can be used as a guide when designing a reaction mixture.
- Smaller volumes are more susceptible to pipetting errors.
- An important point to keep in mind for a successful digestion is mixing. Ensure thorough mixing for complete digestion. GeNei™ recommends gentle up and down pipetting of reaction mixture followed by a short spin in microcentrifuge.
- Incubation time may be shortened if an excess of restriction enzyme is added to the reaction mixture and vice versa.
- Enzymes should be diluted (if required) using respective dilution buffer. The diluted enzymes should be used the same day.
- The termination of the reaction may be done either by adding stop solution or by heat inactivation at 65°C for 20 minutes. Phenol-chloroform extraction may be followed as an alternative method for the restriction enzymes that cannot be heat inactivated.

## Factors influencing restriction enzyme activity

### Nature of DNA:

The nature of the substrate strongly influences the activity of restriction enzymes. The most important parameters are:

- base distribution in natural DNA
- tertiary structure of DNA
- base composition of the flanking sequence
- the position of the cleavage site with respect to each other.

If the DNA has contaminants like phenol, chloroform, alcohol, detergents, EDTA the restriction enzyme activity will be inhibited.

### Temperature:

Optimal digestion of DNA varies over a wide temperature range for different restriction enzymes. The restriction enzymes supplied by Bangalore Genei include enzymes for which the optimum incubation temperature is different from the standard incubation temperature of 37° C. These enzymes are listed below.

Enzymes	Assay Temperature
<i>Apa</i> I	25° C
<i>Bcl</i> I	50° C
<i>Bst</i> E II	60° C
<i>Sfi</i> I	50° C
<i>Sma</i> I	25° C
<i>Taq</i> I	65° C

### Buffer System:

Tris-HCl is the most commonly used buffering agent in incubation mixtures. This buffer system is markedly temperature dependent. The change in pH per 10° C amounts to approx 0.3.

Another important factor affecting the optimum activity is the appropriate ionic environment. Mg<sup>2+</sup> ions are an absolute requirement for all restriction enzymes, whereas the addition of other salt components depends on the different nucleases. Sometimes the presence of BSA in the reaction mix has the crucial influence on the activity of enzymes, because it stabilizes the enzyme, binds some impurities, prevents the enzyme adsorption to the test tube surface.

### Methylation of DNA:

Restriction endonucleases are part of prokaryotic restriction/modification systems. The digestion of DNA isolated during cloning steps in bacterial cells can be strongly affected by the methylation of specific adenosine or cytosine residues in the recognition sequence of the restriction enzyme of interest.

Many *E.coli* host strains possess two nucleotide sequence specific methylases; the *dam* methylase which modifies adenine residues to N<sup>6</sup>-methyladenine in the sequence GATC and the *dcm* methylase which modifies the internal cytosine residues to 5-methylcytosine in CCAGG or CCTGG sequences. Digestion of DNA may be inhibited by *dam* or *dcm* methylation sequences.

Restriction enzymes affected by *dam* and *dcm* methylation are listed below.

Enzymes inhibited by <i>dam</i> methylation		Enzymes not inhibited by <i>dam</i> methylation	
<i>Bcl</i> I	T/ <u>GATCA</u> <sup>+</sup>	<i>Bam</i> H I	G/ <u>GATCC</u>
<i>Cla</i> I	AT/ <u>CGAT</u> <sup>+</sup>	<i>Bgl</i> II	A/ <u>GATCT</u>
<i>Mbo</i> II	GAAGA(N) <sub>8/7</sub> <sup>+</sup>	<i>Pvu</i> I	CG <u>AT</u> /CG
<i>Mbo</i> I	/ <u>GATC</u> <sup>+</sup>	<i>Sau</i> 3A I	/ <u>GATC</u>
<i>Nru</i> I	TCG/ <u>CGA</u> <sup>+</sup>		
<i>Taq</i> I	T/ <u>CGA</u> <sup>+</sup>		
<i>Xba</i> I	T/ <u>CTAGA</u> <sup>+</sup>		

Enzymes inhibited by <i>dcm</i> methylation		Enzymes not inhibited by <i>dcm</i> methylation	
<i>Stu</i> I	AGG/ <u>CCT</u> <sup>+</sup>	<i>Bam</i> H I	G/GAT <u>C</u> <sup>o</sup>
		<i>Bgl</i> I	<u>GCC(N)<sub>4</sub></u> <sup>o</sup> /NGGC
		<i>Hae</i> III	GG/ <u>CC</u> <sup>o</sup>
		<i>Kpn</i> I	GGTAC/ <u>C</u> <sup>o</sup>
		<i>Nar</i> I	GG/ <u>CGCC</u> <sup>o</sup>
		<i>Sfi</i> I	GG <u>CC(N)<sub>4</sub></u> <sup>o</sup> /NG <u>CC</u> <sup>o</sup>

### Reaction Buffer for Restriction Enzymes

Bangalore Genei provides colour coded 10X assay buffer with each restriction enzyme to ensure optimal activity. Some restriction enzymes require BSA at a final concentration of 100 µg/ml for optimal activity. BSA is supplied as 10 mg/ml (100 X) stock when required and should be added to the reaction mixture.

#### Final Concentration in mM (1X Recipe)

Buffer	Tris HCl	Tris - Acetate	Sodium Chloride	Magnesium Chloride	Magnesium Acetate	Potassium Acetate	DTT	pH	Enzymes
A	10	—	150	7	—	—	1	7.9	<i>EcoR V</i> , <i>Not I*</i> , <i>Sal I</i>
B	10	—	100	10	—	—	1	8.0	<i>BamH I</i> , <i>Bgl I</i> , <i>BstE II</i> , <i>Bcl I</i> , <i>Bgl II</i> , <i>Hinc II</i> , <i>Mbo I</i> , <i>Mlu I</i> , <i>Nru I</i> , <i>Nsi I</i> , <i>Pst I</i> , <i>Pvu I</i> , <i>Ssp I</i> , <i>Taq I</i> .
C	10	—	50	10	—	—	1	7.8	<i>Alu I</i> , <i>Hae III</i> , <i>Hind III</i> , <i>Hinf I</i> , <i>Msp I</i> , <i>Nhe I</i> , <i>Pvu II</i> , <i>Spe I</i> , <i>Stu I</i> , <i>Xba I</i> .
L	10	—	—	10	—	—	1	7.4	<i>Hpa II</i> , <i>Kpn I</i> , <i>Sac I</i> , <i>Xma I</i>
E	—	33	—	—	10	66	0.5	7.9	<i>Acc I</i> , <i>Apa I</i> , <i>Ase I</i> , <i>Ava I</i> , <i>Ban I</i> , <i>Cla I</i> , <i>Dra I</i> , <i>Hha I</i> , <i>Hpa I</i> , <i>Nae I</i> , <i>Nar I</i> , <i>Nco I</i> , <i>Sau3A I</i> , <i>Sau96 I</i> , <i>SnaB I</i> , <i>Sfi I</i> , <i>Sma I</i> , <i>Xho I</i> , <i>Xmn I</i> .

- Note:** 1. *EcoR I* has unique Buffer: 50 mM Tris HCl (pH 8.0) 100 mM NaCl, 10 mM MgCl<sub>2</sub> and 5 mM β-Mercaptoethanol,  
 2. *Not I\** Buffer A with 0.01% Triton X 100.  
 3. The enzymes printed **bold** need BSA for optimum activity.

### Diluent Buffers

Diluent buffers are for diluting the restriction enzymes when needed. Buffer composition and the list of restriction enzymes with its appropriate dilution buffer are given below:

Diluent Buffer 1	Enzymes				
10 mM Tris-HCl (pH 7.4), 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 200 µg/ml Nuclease free BSA and 50% glycerol.	<i>Acc I</i>	<i>Cla I</i>	<i>Hpa II</i>	<i>Nhe I</i>	<i>SnaB I</i>
	<i>Apa I</i>	<i>Dra I</i>	<i>Kpn I</i>	<i>Nru I</i>	<i>Ssp I</i>
	<i>Ase I</i>	<i>EcoR V</i>	<i>Mbo I</i>	<i>Nsi I</i>	<i>Spe I</i>
	<i>Ava I</i>	<i>Hae III</i>	<i>Mlu I</i>	<i>Pvu II</i>	<i>Stu I</i>
	<i>Ban I</i>	<i>Hha I</i>	<i>Msp I</i>	<i>Sau96 I</i>	<i>Xho I</i>
	<i>Bcl I</i>	<i>Hinc II</i>	<i>Nae I</i>	<i>Sau3A I</i>	<i>Xma I</i>
	<i>BstE II</i>	<i>Hinf I</i>	<i>Nar I</i>	<i>Sal I</i>	<i>Xmn I</i>
		<i>Hpa I</i>	<i>Nco I</i>	<i>Sma I</i>	
	<b>Diluent Buffer 2</b>				
	10 mM Tris-HCl (pH 7.4), 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 500 µg/ml Nuclease Free BSA and 50% glycerol.	<i>Alu I</i>	<i>Bgl I</i>	<i>Bgl II</i>	
<i>Hind III</i>		<i>Pvu I</i>	<i>Sac I</i>	<i>Taq I</i>	
<b>Diluent Buffer 3</b>					
10 mM Tris-HCl (pH 7.4), 250 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 200 µg/ml Nuclease free BSA, 0.15 % Triton X-100 and 50% glycerol.	<i>BamH I</i>	<i>EcoR I</i>	<i>Not I</i>	<i>Pst I</i>	
	<i>Sfi I</i>	<i>Xba I</i>			

TOOLS FOR GENOMIC RESEARCH

## Relative Activity of Restriction Enzymes in Bangalore Genei Assay Buffer System

Sl.No.	Restriction Enzymes	10X Assay Buffers				
		A	B	C	E	L
1.	<i>Acc I</i>	0	0	50	<b>100</b>	25
2.	<i>Alu I</i>	50	75	<b>100</b>	100	100
3.	<i>Apa I</i>	0	0	50	<b>100</b>	50
4.	<i>Ase I</i>	50	100	60	<b>100</b>	0
5.	<i>Ava I</i>	25	25	50	<b>100</b>	25
6.	<i>BamH I</i>	50	<b>100</b>	100	100	100
7.	<i>Bcl I</i>	50	<b>100</b>	100	100	75
8.	<i>Bgl I</i>	100	<b>100</b>	75	25	0
9.	<i>Bgl II</i>	75	<b>100</b>	75	25	0
10.	<i>BstE II</i>	25	<b>100</b>	50	100	0
11.	<i>Cla I</i>	25	50	50	<b>100</b>	75
12.	<i>Dra I</i>	50	50	50	<b>100</b>	75
13.	<i>EcoR I</i>	75	100	100	100	25
14.	<i>EcoR V</i>	<b>100</b>	75	50	50	0
15.	<i>Hae III</i>	75	100	<b>100</b>	100	25
16.	<i>Hha I</i>	75	100	100	<b>100</b>	50
17.	<i>Hinc II</i>	50	<b>100</b>	50	100	0
18.	<i>Hind III</i>	0	25	<b>100</b>	100	50
19.	<i>Hinf I</i>	100	100	<b>100</b>	50	50
20.	<i>Hpa I</i>	0	0	0	<b>100</b>	25
21.	<i>Hpa II</i>	0	25	50	75	<b>100</b>
22.	<i>Kpn I</i>	0	0	75	50	<b>100</b>
23.	<i>Mbo I</i>	50	<b>100</b>	50	50	0
24.	<i>Mlu I</i>	100	<b>100</b>	50	25	0
25.	<i>Msp I</i>	25	75	<b>100</b>	100	100

## Relative Activity of Restriction Enzymes in Bangalore Genei Assay Buffer System

Sl.No.	Restriction Enzymes	10X Assay Buffers				
		A	B	C	E	L
26.	<i>Nae</i> I	0	25	50	<b>100</b>	100
27.	<i>Nar</i> I	0	25	50	<b>100</b>	50
28.	<i>Nco</i> I	50	100	100	<b>100</b>	50
29.	<i>Not</i> I	<b>100</b>	100	75	0	0
30.	<i>Nhe</i> I	0	0	<b>100</b>	100	100
31.	<i>Nsi</i> I	100	<b>100</b>	75	75	50
32.	<i>Nru</i> I	100	<b>100</b>	0	100	0
33.	<i>Pst</i> I	100	<b>100</b>	100	50	100
34.	<i>Pvu</i> I	100	<b>100</b>	50	75	0
35.	<i>Pvu</i> II	0	25	<b>100</b>	50	25
36.	<i>Sac</i> I	25	25	50	100	<b>100</b>
37.	<i>Sal</i> I	<b>100</b>	50	0	0	0
38.	<i>Sau</i> 3A I	25	25	25	<b>100</b>	75
39.	<i>Sau</i> 96 I	100	100	75	<b>100</b>	75
40.	<i>Sfi</i> I	50	50	0	<b>100</b>	0
41.	<i>Sma</i> I	0	0	0	<b>100</b>	0
42.	<i>Sna</i> B I	0	0	30	<b>100</b>	0
43.	<i>Spe</i> I	50	50	<b>100</b>	100	100
44.	<i>Ssp</i> I	25	<b>100</b>	100	100	0
45.	<i>Stu</i> I	25	75	<b>100</b>	100	25
46.	<i>Taq</i> I	25	<b>100</b>	75	100	75
47.	<i>Xba</i> I	0	0	<b>100</b>	100	0
48.	<i>Xho</i> I	100	100	100	<b>100</b>	25
49.	<i>Xma</i> I	0	0	100	100	<b>100</b>

## NOTE:

- Bangalore Genei supplies the buffers that are typed in italics and bold with the respective enzymes.
- In case of *Eco*R I, the percentage activity is reported with respect to the unique buffer supplied
- Chart serves as a guide to choose the compatible buffer for double digestion.

Product	Sequence	Assay Condition		Volume Activity (Units/μl)	New Cat No.	Old Cat No.	Pack Size	Price (Rs.)
		Buffer	Temp.					
<i>Acc I</i>	<i>GT↓A(C)T(G)AC</i>	E	37°C	1-5	105787	MBE43S	80 U	1500
				1-5	105786	MBE43L	400 U	4000
<i>Alu I</i>	<i>AG↓CT</i>	C	37°C	5-10	105727	MBE17S	100 U	1450
				5-10	105726	MBE17L	500 U	4400
<i>Ase I</i> <b>New</b>	<i>AT↓TAAT</i>	E	37°C	1-5	107557	MBE60S	100 U	1250
					107558	MBE60L	500 U	4500
<i>Apa I</i>	<i>GGG C↓C</i>	E	25°C	5-10	105808	MBE53S	800 U	950
				5-10	105807	MBE53L	4000 U	3450
<i>Ava I</i>	<i>C↓PyCGPuG</i>	E	37°C	5-10	105791	MBE45S	200 U	1600
				5-10	105790	MBE45L	1000 U	4100
<i>BamH I</i>	<i>G↓GATCC</i>	B+ BSA	37°C	10	105735	MBE1S	2000 U	850
				10	105734	MBE1M	4000 U	1400
				10	105732	MBE1L	10000 U	3100
				High Conc.	40-100	105733	MBE1LC	10000 U
<i>Ban I</i>	<i>G↓GPuPCC</i>	E	37°C	5-10	105812	MBE55S	80 U	1400
				5-10	105811	MBE55L	400 U	3450
<i>Bcl I</i>	<i>T↓GATCA</i>	B	50°C	10	105764	MBE32S	200 U	1100
				10	105762	MBE32L	1000 U	2900
				High Conc.	40-100	105763	MBE32LC	1000 U
<i>Bgl I</i>	<i>GCCNMMN↓NGGC</i>	B	37°	10	105829	MBE8S	800 U	1750
				10	105827	MBE8L	4000 U	4100
				High Conc.	40-100	105828	MBE8LC	4000 U
<i>Bgl II</i>	<i>A↓GATCT</i>	B	37°C	10	105757	MBE2S	200 U	1300
				10	105755	MBE2L	1000 U	3000
				High Conc.	40-100	105756	MBE2LC	1000 U
<i>BstE II</i>	<i>G↓GTNACC</i>	B	60°C	10	105785	MBE42S	200 U	1400
				10	105784	MBE42L	1000 U	3450
<i>Cla I</i>	<i>AT↓CGAT</i>	E+ BSA	37°C	10	105750	MBE25S	200 U	1500
				10	105749	MBE25L	1000 U	3300
<i>Dra I</i>	<i>TTT↓AAA</i>	E	37°C	10	105770	MBE35S	400 U	1100
				10	105769	MBE35L	2000 U	2650

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Product	Sequence	Assay Condition		Volume Activity (Units/μl)	New Cat No.	Old Cat No.	Pack Size	Price (Rs.)
		Buffer	Temp.					
<i>EcoR I</i>	<i>G↓AATTC</i>	Unique Buffer	37° C	20	105779	MBE3S	4000 U	850
				20	105777	MBE3L	20000 U	2650
				40-100	105778	MBE3LC	20000 U	2650
<i>EcoR V</i>	<i>GAT↓ATC</i>	A+ BSA	37° C	10	105832	MBE9S	1200 U	1300
				10	105830	MBE9L	6000 U	2750
				40-100	105831	MBE9LC	6000 U	2750
<i>Hae III</i>	<i>GG↓CC</i>	C	37° C	10	105709	MBE10S	800 U	1400
				10	105707	MBE10L	4000 U	3650
				40-100	105708	MBE10LC	4000 U	3650
<i>Hha I</i>	<i>GCG↓C</i>	E+ BSA	37° C		105721	MBE14S	400 U	1500
					105719	MBE14L	2000 U	3200
				40-100	105720	MBE14LC	2000 U	3200
<i>Hinc II</i>	<i>GT(T/C)↓(A/G)AC</i>	B+ BSA	37° C		105731	MBE19S	200 U	1600
					105730	MBE19L	1000 U	4100
<i>Hind III</i>	<i>A↓AGCTT</i>	C	37° C	20	105824	MBE6S	4000 U	1000
				20	105823	MBE6L	20000 U	3550
<i>Hinf I</i>	<i>G↓ANTC</i>	C	37° C	10	105740	MBE21S	2000 U	1750
				10	105738	MBE21L	10000 U	4100
				40-100	105739	MBE21LC	10000 U	4100
<i>Hpa I</i>	<i>GTT↓AAC</i>	E	37° C	3-10	105754	MBE29S	100 U	1400
				3-10	105753	MBE29L	500 U	3750
<i>Hpa II</i>	<i>C↓CGG</i>	L	37° C	10	105737	MBE20S	200 U	1300
				10	105736	MBE20L	1000 U	3550
<i>Kpn I</i>	<i>GGTAC↓C</i>	L+ BSA	37° C	10	105743	MBE22S	800 U	1300
				10	105741	MBE22L	4000 U	2900
				40-100	105742	MBE22LC	4000 U	2900

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Product	Sequence	Assay Condition		Volume Activity (Units/μl)	New Cat No.	Old Cat No.	Pack Size	Price (Rs.)
		Buffer	Temp.					
<i>Mbo</i> I	↓GATC	B	37°C	5	105752	MBE27S	80 U	1750
				5	105751	MBE27L	400 U	4300
<i>Mlu</i> I	A↓CGCGT	B	37°C	5-10	105759	MBE30S	400 U	1500
				5-10	105758	MBE30L	2000 U	3300
<i>Msp</i> I	C↓CGG	C	37°C	10	105761	MBE31S	400 U	1200
				10	105760	MBE31L	2000 U	2750
<i>Nae</i> I	GCC↓GGC	E + BSA	37°C	10	105797	MBE49S	80 U	1250
				10	105796	MBE49L	400 U	3550
<i>Nar</i> I	GG↓CGCC	E	37°C	10	105802	MBE50S	80 U	1250
				10	105801	MBE50L	400 U	3550
<i>Nco</i> I	C↓CATGG	E	37°C	5-10	105723	MBE15S	80 U	1750
				5-10	105722	MBE15L	400 U	4000
<i>Not</i> I	GC↓GGCCGC	A + BSA	37°C	5-10	105725	MBE16S	80 U	1750
				5-10	105724	MBE16L	400 U	4100
<i>Nhe</i> I	G↓CTAGC	C + BSA	37°C	5-10	105772	MBE37S	100 U	1500
				5-10	105771	MBE37L	500 U	3300
<i>Nsi</i> I	ATGCA↓T	B	37°C	10	105804	MBE51S	80 U	1250
				10	105803	MBE51L	400 U	3550
<i>Nru</i> I	TCG↓CGA	B	37°C	5-10	105776	MBE39S	100 U	1600
				5-10	105775	MBE39L	500 U	4300

All restriction enzymes and 10X assay buffers should be stored at -20°C

Product	Sequence	Assay Condition		Volume Activity (Units/μl)	New Cat No.	Old Cat No.	Pack Size	Price (Rs.)
		Buffer	Temp.					
<i>Pst</i> I	CTGCA↓G	B	37°C	10	105716	MBE12S	1200 U	1200
				10	105715	MBE12M	3000 U	1400
				10	105713	MBE 12L	6000 U	2750
				40-100	105714	MBE12LC	6000 U	2750
High Conc.								
<i>Pvu</i> I	CGAT↓CG	B	37°C	5	105766	MBE33S	40 U	1800
				5	105765	MBE33L	200 U	4850
<i>Pvu</i> II	CAG↓CTG	C	37°C	10	105712	MBE11S	400 U	1200
				10	105710	MBE11L	2000 U	2450
				40-100	105711	MBE11LC	2000 U	2450
High Conc.								
<i>Sac</i> I	GAGCT↓C	L+ BSA	37°C	10	105729	MBE18S	400 U	1750
				10	105728	MBE18L	2000 U	4100
<i>Sal</i> I	G↓TCGAC	A+ BSA	37°C	10	105800	MBE4S	400 U	1300
				10	105798	MBE4L	2000 U	2900
				40-100	105799	MBE4LC	2000 U	2900
High Conc.								
<i>Sau</i> 3A I	↓GATC	E+ BSA	37°C	5-10	105822	MBE5S	80 U	1850
				5-10	105821	MBE5L	400 U	4550
<i>Sau</i> 96 I	G↓G NCC	E	37°C	5-10	105810	MBE54S	100 U	1350
				5-10	105809	MBE54L	500 U	3650
<i>Sfi</i> I	GGCCNNNN↓NGGCC	E+ BSA	50°C	5-10	105781	MBE40S	50 U	1200
				5-10	105780	MBE40L	250 U	2950
<i>Sma</i> I	CCC↓GGG	E	25°C	5-10	105718	MBE13S	200 U	1400
				5-10	105717	MBE13L	1000 U	3200
<i>Sna</i> B I	New TAC↓GTA	E	37°C	1-5	107559	MBE61S	100 U	1250

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Product	Sequence	Assay Condition		Volume Activity (Units/μl)	New Cat No.	Old Cat No.	Pack Size	Price (Rs.)
		Buffer	Temp.					
<i>Spe I</i>	A↓CTAGT	C+ BSA	37° C	1-5	105774	MBE38S	80 U	1450
				1-5	105773	MBE38L	400 U	3850
<i>Ssp I</i>	AAT↓ATT	B+ BSA	37° C	5-10	105783	MBE41S	100 U	1300
				5-10	105782	MBE41L	500 U	3000
<i>Stu I</i>	AGG↓CCT	C	37° C	10	105789	MBE44S	400 U	1200
				10	105788	MBE44L	2000 U	3100
<i>Taq I</i>	T↓CGA	B+ BSA	65° C	10	105826	MBE7S	800 U	1600
				10	105825	MBE7L	4000 U	4400
<i>Xba I</i>	T↓CTAGA	C+ BSA	37° C	10	105746	MBE23S	1000 U	1400
				10	105744	MBE23L	5000 U	3750
				High Conc.	40-100	105745	MBE23LC	5000 U
<i>Xho I</i>	C↓TCGAG	E+ BSA	37° C	10	105748	MBE24S	1000 U	1200
				10	105747	MBE24L	5000 U	2900
<i>Xma I</i>	C↓CCGGG	L+ BSA	37° C	1-5	105768	MBE34L	50 U	1300
				1-5	105767	MBE34J	250 U	3750
<i>Xmn I</i>	GAANM↓NNTTC	E	37° C	5-10	105814	MBE56S	100 U	1400
				5-10	105813	MBE56L	500 U	3450

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## Reaction Buffer Set

Genei also caters to your additional requirement of reaction buffers.

### Ordering Information:

Product	Size	New Cat #	Old Cat #	Price (Rs)
Buffer Set A, B, C, L, E & BSA	1 ml each	105986	REB01S	1650
Buffer Set A Buffer A BSA	4 x 1 ml 1 x 1 ml	105981	REB01A	1100
Buffer Set B Buffer B BSA	4 x 1 ml 1 x 1 ml	105982	REB01B	1100
Buffer Set C Buffer C BSA	4 x 1 ml 1 x 1 ml	105983	REB01C	1100
Buffer Set L Buffer L BSA	4 x 1 ml 1 x 1 ml	105985	REB01L	1100
Buffer Set E Buffer E BSA	4 x 1 ml 1 x 1 ml	105984	REB01E	1100

## Diluent Buffers

### Ordering Information:

Product	Size	New Cat #	Old Cat #	Price (Rs)
Diluent Buffer set Buffer 1 Buffer 2 Buffer 3	3 x 1 ml 1 x 1 ml 1 x 1 ml	105990	REDB02S	1650
Diluent Buffer 1	3 x 1 ml	105987	REDB021	1100
Diluent Buffer 2	3 x 1 ml	105988	REDB022	1100
Diluent Buffer 3	3 x 1 ml	105989	REDB023	1100