

## 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 sub-optimally 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 λ *dam*- DNA, pBR 322 DNA, Ad2 DNA, λ / *EcoR* I digest or λ / *Hind* III digest.

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

#### Overnight Non-specific Nuclease Assay:

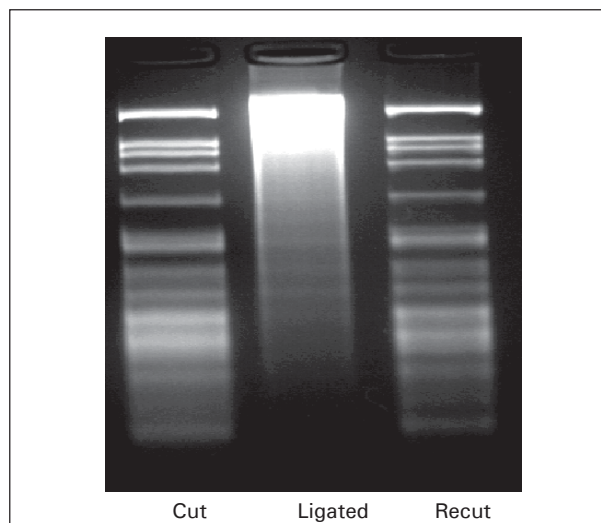
Every preparation of restriction enzyme is tested for non-specific nucleases. Varying units of enzyme is incubated with 1 µg of substrate DNA at the recommended assay conditions in 50 µl reaction volume for 16 - 20 hours. A sharp unaltered banding pattern is an indication of the purity of enzyme free 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 re-cleavage 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.

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## Tips to use Restriction Enzymes

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- 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>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>Cla</i> I                                | AT/CGAT <sup>+</sup>                 | <i>Bam</i> HI                                   | G/G <sup>o</sup> ATCC |
| <i>Mbo</i> II                               | GAAGA(N) <sub>8/7</sub> <sup>+</sup> | <i>Bgl</i> II                                   | A/G <sup>o</sup> ATCT |
| <i>Mbo</i> I                                | /GATC <sup>+</sup>                   | <i>Pvu</i> I                                    | CG <sup>o</sup> AT/CG |
| <i>Nru</i> I                                | TCG/CGA <sup>+</sup>                 | <i>Sau</i> 3A I                                 | /G <sup>o</sup> ATC   |
| <i>Taq</i> I                                | T/CGA <sup>+</sup>                   |   |                       |
| <i>Xba</i> I                                | T/CTAGA <sup>+</sup>                 |   |                       |

| Enzymes inhibited by <i>dcm</i> methylation |                      | Enzymes not inhibited by <i>dcm</i> methylation |  |
|---|----------------------|---|--|
| <i>Stu</i> I                                | AGG/CCT <sup>+</sup> | <i>Bam</i> HI                                   | G/GATCC <sup>o</sup>                     |
|   |                      | <i>Bgl</i> I                                    | GCC(N) <sub>4</sub> /NGGC <sup>o</sup>   |
|   |                      | <i>Hae</i> III                                  | GG/CC <sup>o</sup>                       |
|   |                      | <i>Kpn</i> I                                    | GGTAC/C <sup>o</sup>                     |
|   |                      | <i>Nar</i> I                                    | GG/CGCC <sup>o</sup>                     |
|   |                      | <i>Sfi</i> I                                    | GGCC(N) <sub>4</sub> /NGGCC <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 | Potassium Chloride | DTT | pH           | Enzymes  |
|--------|-----------------|----------------|-----------------|--------------------|-------------------|-------------------|--------------------|-----|--------------|--|
| A      | 10              | —              | 150             | 7                  | —                 | —                 | —                  | 1   | 7.9          | <b>EcoRV</b> , <b>Not I*</b> , <b>Sal I</b>  |
| B      | 10              | —              | 100             | 10                 | —                 | —                 | —                  | 1   | 8.0          | <b>Bgl I</b> , <b>Bgl II</b> , <b>Hinc II</b> , <b>Mbo I</b> , <b>Mlu I</b> , <b>Pvu I</b> , <b>Ssp I</b> .  |
| C      | 10              | —              | 50              | 10                 | —                 | —                 | —                  | 1   | 7.8          | <b>Alu I</b> , <b>Hae III</b> , <b>Hinf I</b> , <b>Msp I</b> , <b>Nhe I</b> , <b>Pvu II</b> , <b>Spe I</b> , <b>Stu I</b> , <b>Xba I</b> .   |
| D      | Optimised Conc. | —              | —               | 10                 | —                 | —                 | Optimised Conc.    | —   | Optimised pH | <b>BamH I</b> , <b>EcoR I</b> , <b>Hind III</b> , <b>Mlu I</b> , <b>Nsi I</b> , <b>Taq I</b> , <b>Pst I</b> , <b>BstE II</b> , <b>Nde I</b> , <b>Nru I</b>   |
| L      | 10              | —              | —               | 10                 | —                 | —                 | —                  | 1   | 7.4          | <b>Hpa II</b> , <b>Kpn I</b> , <b>Sac I</b> , <b>Xma I</b>   |
| E      | —               | 33             | —               | —                  | 10                | 66                | —                  | 0.5 | 7.9          | <b>Apa I</b> , <b>Ava I</b> , <b>Cla I</b> , <b>Dra I</b> , <b>Hha I</b> , <b>Hpa I</b> , <b>Nae I</b> , <b>Nco I</b> , <b>Sau3A I</b> , <b>SnaB I</b> , <b>Sfi I</b> , <b>Sma I</b> , <b>Xho I</b> , <b>Xmn I</b> . |

#### Note:

1. **Not I\*** Buffer A with 0.01% Triton X 100.
2. 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 (pH 7.4),<br>50 mM KCl, 0.1 mM EDTA,<br>1 mM DTT, 200 µg/ml<br>Nuclease free BSA, 0.1% Triton<br>and 50% glycerol.   | <b>Apa I</b>    | <b>Hae III</b> | <b>Mbo I</b> | <b>Nsi I</b>   | <b>Spe I</b>  |
|   | <b>Alu I</b>    | <b>Hha I</b>   | <b>Mlu I</b> | <b>Pvu I</b>   | <b>Ssp I</b>  |
|   | <b>Ava I</b>    | <b>Hinc II</b> | <b>Msp I</b> | <b>Pvu II</b>  | <b>Stu I</b>  |
|   | <b>BamH I</b>   | <b>Hinf I</b>  | <b>Nae I</b> | <b>Sac I</b>   | <b>Xba I</b>  |
|   | <b>BstE II</b>  | <b>Hpa I</b>   | <b>Nhe I</b> | <b>Sau3A I</b> | <b>Xho I</b>  |
|   | <b>Cla I</b>    | <b>Hpa II</b>  | <b>Nco I</b> | <b>Sal I</b>   | <b>Xma I</b>  |
|   | <b>Dra I</b>    | <b>Kpn I</b>   | <b>Not I</b> | <b>Sma I</b>   | <b>Xmn I</b>  |
| Diluent Buffer 2  |                 |                |              |                |               |
| 10 mM Tris (pH 7.4), 300 mM NaCl, 0.1 mM EDTA,<br>1 mM DTT, 500 µg/ml Nuclease Free BSA, 0.1%<br>Triton and 50% glycerol.       | <b>Hind III</b> | <b>Taq I</b>   | <b>EcoRV</b> |                |               |
| Diluent Buffer 3  |                 |                |              |                |               |
| 10 mM Tris-HCl (pH 7.4), 250 mM NaCl, 0.1 mM<br>EDTA, 1 mM DTT, 200 µg/ml Nuclease free BSA,<br>0.1% Triton X and 50% glycerol. | <b>EcoRI</b>    | <b>Pst I</b>   | <b>Sfi I</b> | <b>Bgl I</b>   | <b>Bgl II</b> |