

EcoRV

Sequence of EcoRV: 5'...GATATC...3' 3'...CTATAG...5'

| Catalog-no | Description | units |
|------------|-------------|----------|
| 250-115S | EcoR V | 3000 u |
| 250-115L | EcoR V | 5x3000 u |

Overhang: 5' - **blunt**

Cut Site:

GAT/ATC
CTA/TAG

Isoschizomers: *Eco32 I*

Neoschizomers: N/A

Source: Escherichia coli, J62plg 74.

Buffer supplied: 10x M

Substrate for unit definition: λ DNA

Reaction conditions: 50 mM NaCl, 10 mM Tris-HCl (pH 7.9 at 25°C), 10 mM MgCl₂, 1 mM dithiothreitol, 100 μ g/ml BSA. **Incubate at 37°C**

Storage buffer: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 μ g/ml BSA and 50% glycerol

Store: at -20°C.

Absence of contaminants: Fifty units of EcoRV do not produce any unspecific cleavage products after 16 hrs incubation with 1 μ g of λ DNA at 37°C. After 20-fold overdigestion with EcoRV, greater than 95% of the DNA fragments can be ligated and recut with this enzyme

Heat inactivation: 80°C for 20 minutes

Star activity: Conditions of low ionic strength, high enzyme concentration, glycerol concentration >5%, or pH >8.0 may result in star activity

Note: Activity may be blocked by overlapping CpG methylation.

Recommended Reaction Conditions

* Requires Triton X-100 for optimal activity.

| Restriction Enzyme | Tris-HCl (Tris-Acetate) {Bis Tris Propane-HCl} | | NaCl (K-Acetate) {KCl} | MgCl ₂ (Mg-Acetate) | DTT | BSA | TX-100 | Temp. | Buffer |
|--------------------|--|-----------|------------------------------|-----------------------------------|-----|-------|--------|-------|------------------|
| | mM | pH (25°C) | mM | mM | mM | µg/ml | % | °C | |
| <i>Alu</i> I | 10 | 7.9 | - | 10 | 1 | 100 | - | 37 | L |
| <i>Apa</i> L I | 10 | 7.9 | - | 10 | 1 | 100 | - | 37 | L |
| <i>Asu</i> II | 10 | 7.9 | 50 | 10 | 1 | 100 | 0.1 | 37 | M* |
| <i>Bam</i> H I | 10 | 7.9 | 100 | 5 | 1 | 100 | - | 37 | U |
| <i>Bcl</i> I | 10 | 7.9 | 50 | 10 | 1 | 100 | - | 50 | M |
| <i>Bgl</i> I | 100 | 7.9 | 50 | 5 | - | 100 | 0.025 | 37 | U |
| <i>Bgl</i> II | 50 | 7.9 | 100 | 10 | 1 | 100 | - | 37 | H |
| <i>Bse</i> A I | 10 | 8.0 | 100 | 5 | 1 | 100 | 0.02 | 55 | U |
| <i>Bse</i> B I | 10 | 7.9 | 50 | 10 | 1 | 100 | - | 60 | M |
| <i>Bse</i> C I | 50 | 7.9 | 100 | 10 | 1 | 100 | - | 55 | H |
| <i>Bsh</i> F I | (20) | 7.9 | (50) | (10) | 1 | 100 | - | 37 | A |
| <i>Bsi</i> S I | (33) | 7.9 | (66) | (10) | 0.5 | 100 | 0.1 | 55 | U |
| <i>Bss</i> A I | 20 | 8.5 | {100} | 3 | - | 100 | 0.04 | 65 | U |
| <i>Bst</i> E I | 10 | 7.4 | {100} | 5 | 1 | 100 | 0.1 | 60 | U |
| <i>Csp</i> A I | {10} | 7.0 | - | 10 | 1 | 100 | - | 37 | U |
| <i>Eco</i> R I | 100 | 7.4 | 50 | 5 | - | 100 | 0.025 | 37 | U |
| <i>Eco</i> R V | 10 | 7.9 | 50 | 10 | 1 | 100 | - | 37 | M |
| <i>Hind</i> III | 10 | 7.9 | 50 | 10 | 1 | 100 | - | 37 | M |
| <i>Hinf</i> I | 50 | 7.9 | 100 | 10 | 1 | 100 | - | 37 | H |
| <i>Hpa</i> I | (20) | 7.9 | (50) | (10) | 1 | 100 | - | 37 | A |
| <i>Kpn</i> I | 10 | 7.0 | - | 10 | 1 | 100 | 0.01 | 37 | U |
| <i>Mbo</i> I | 10 | 8.0 | {100} | 10 | 1 | 100 | - | 37 | U |
| <i>Msp</i> C I | 10 | 7.9 | 150 | 10 | 1 | 100 | - | 37 | SH |
| <i>Nae</i> I | 10 | 7.9 | - | 10 | 1 | 100 | - | 37 | L |
| <i>Nco</i> I | 50 | 7.9 | 100 | 10 | 1 | 100 | 0.02 | 37 | H* |
| <i>Nhe</i> I | (20) | 7.9 | (50) | (10) | 1 | 100 | - | 37 | A |
| <i>Not</i> I | 50 | 7.9 | 100 | 5 | 1 | 100 | - | 37 | U |
| <i>Nru</i> I | 50 | 8.0 | {100} | 10 | - | 100 | - | 37 | U |
| <i>Psp</i> P I | 10 | 7.9 | 50 | 10 | 1 | 100 | - | 25 | M |
| <i>Pst</i> I | 50 | 7.4 | 100 | 10 | 1 | 100 | - | 37 | U |
| <i>Pvu</i> II | 10 | 7.9 | 50 | 10 | 1 | 100 | - | 37 | M |
| <i>Rsa</i> I | 10 | 7.9 | 50 | 10 | 1 | 100 | - | 37 | M |
| <i>Sal</i> I | 10 | 7.9 | 150 | 10 | 1 | 100 | - | 37 | SH |
| <i>Sca</i> I | 10 | 7.4 | 100 | 10 | 1 | 100 | - | 37 | U |
| <i>Sfi</i> I | 10 | 7.9 | 50 | 10 | 1 | 100 | - | 50 | M |
| <i>Sgr</i> B I | 10 | 7.9 | - | 10 | 1 | 100 | 0.1 | 37 | L* |
| <i>Sla</i> I | 10 | 7.9 | 150 | 10 | 1 | 100 | - | 37 | SH |
| <i>Sma</i> I | (20) | 7.9 | (50) | (10) | 1 | 100 | - | 25 | A |
| <i>Sna</i> B I | {10} | 7.0 | - | 10 | 1 | 100 | - | 37 | U |
| <i>Sph</i> I | 10 | 7.9 | 50 | 10 | 1 | 100 | - | 37 | M |
| <i>Sse</i> B I | 50 | 7.9 | 100 | 10 | 1 | 100 | - | 37 | H |
| <i>Ssp</i> I | 50 | 7.9 | 100 | 10 | 1 | 100 | - | 37 | H |
| <i>Sst</i> I | 10 | 7.9 | - | 10 | 1 | 100 | - | 37 | L |
| <i>Sty</i> I | 50 | 7.9 | 100 | 10 | 1 | 100 | - | 37 | H |
| <i>Taq</i> I | 20 | 8.5 | {100} | 3 | - | 100 | 0.04 | 65 | U |
| <i>Xba</i> I | 10 | 7.9 | 50 | 10 | 1 | 100 | - | 37 | M <i>Sau</i> 3AI |
| | 10 | 7.9 | 50 | 10 | 1 | 100 | - | 37 | M |

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Contact Germany Phone +49-(0)-621- 5720 864 Fax: +49-(0)-621-5724 462

E-Mail: <mailto:info@geneon.net> WEB: <http://www.GeneOn.net>

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Relative Activity of Restriction Enzymes in Reactions Buffers

This table lists relative activities of each restriction enzyme with each buffer assuming the activity of the enzyme under optimal conditions to be 100%.

| Restriction enzyme | Recommended buffer | Enzyme activity (%) | | | | |
|-----------------------|--------------------|---------------------|------------|------------|------------|------------|
| | | L | M | H | SH | A |
| <i>Alu I</i> | L | 100 | 100 | 75 | 10-25 | 75 |
| <i>ApaI</i> | L | 100 | 100 | 10 | <10 | 10-25 |
| <i>Asu II</i> | M* | 75 | 100 | 50-75 | 25 | 50 |
| <i>BamH I</i> | U | 75 | 75-100 | 100 | 50-75 | 75 |
| <i>Bcl I</i> (50°C) | M | 10-25 | 100 | 75 | 50-75 | 10-25 |
| <i>Bgl I</i> | U | 10-25 | 75-100 | 75-100 | 75-100 | 50 |
| <i>Bgl II</i> | H | 10 | 75 | 100 | 75-100 | 10 |
| <i>BseA I</i> (55°C) | U | 10 | 50 | 75-100 | 50-75 | 10 |
| <i>BseB I</i> (60°C) | M | 10-25 | 100 | 50 | 25-50 | <10 |
| <i>BseC I</i> (55°C) | H | 10 | 50 | 100 | 75-100 | 50 |
| <i>BshF I</i> | A | 50-75 | 75-100 | 75 | 50-75 | 100 |
| <i>BsiS I</i> (55°C) | U | 25 | 50 | 25 | 10-25 | 100 |
| <i>BssA I</i> (65°C) | U | 10 | 25 | 75 | 50 | 25 |
| <i>CspA I</i> | U | 50 | <10 | <10 | <10 | <10 |
| <i>EcoR I</i> | U | 25-50 | 50-75 | 75 | 50-75 | 75 |
| <i>EcoR V</i> | M | 10-25 | 100 | 50 | <10 | 75 |
| <i>Hind III</i> | M | 25-50 | 100 | 10-25 | 10-25 | 50 |
| <i>BstE II</i> (60°C) | U | 50 | 50-75 | 75-100 | 50 | 75 |
| <i>Hinf I</i> | H | 10-25 | 50 | 100 | 75-100 | 50 |
| <i>Hpa I</i> | A | 25-50 | 10-25 | 10-25 | 10-25 | 100 |
| <i>Kpn I</i> | U | 75-100 | 25-50 | <10 | <10 | 50 |
| <i>Mbo I</i> | U | 50-100 | 50-100 | 50-100 | 50 | 50-100 |
| <i>MspC I</i> | SH | <10 | 25-50 | 75-100 | 100 | 50 |
| <i>Nae I</i> | L | 100 | 25-50 | 25 | <10 | 50 |
| <i>Nco I</i> | H* | 50-75 | 75-100 | 100 | 100 | 75 |
| <i>Nhe I</i> | A | 100 | 50-75 | 0-20 | <10 | 100 |
| <i>Not I</i> | U | <10 | 25-50 | 75-100 | 75 | 50 |
| <i>Nru I</i> | U | <10 | <10 | 75 | 50-75 | 10 |
| <i>PspP I</i> (25°C) | M | 50-75 | 100 | 50 | 25-50 | 10 |
| <i>Pst I</i> | U | 10-25 | 50-75 | 75-100 | 50-75 | 50 |
| <i>Pvu II</i> | M | 25-50 | 100 | 100 | 25-50 | 50 |
| <i>Rsa I</i> | M | 75-100 | 100 | 50 | <10 | <10 |
| <i>Sal I</i> | SH | <10 | 25-50 | 50 | 100 | <10 |
| <i>Sau3AI</i> | M | 50 | 100 | 50 | <10 | 50 |
| <i>Sca I</i> | U | <10 | 50-75 | 100 | 75-100 | 25 |
| <i>Sfi I</i> (50°C) | M | 75-100 | 100 | 25-50 | 10-25 | 75-100 |
| <i>SgrB I</i> | L* | 75-100 | 75 | 50-75 | 25-50 | <10 |
| <i>Sla I</i> | SH | 25-50 | 75 | 75-100 | 100 | 10-25 |
| <i>Sma I</i> (25°C) | A | <10 | <10 | <10 | <10 | 100 |
| <i>SnaB I</i> | U | 50-75 | 50 | 25 | <10 | 100 |
| <i>Sph I</i> | M | 75-100 | 100 | 50 | 50 | 50 |
| <i>SseB I</i> | H | 50-75 | 75-100 | 100 | 50-75 | 50 |
| <i>Ssp I</i> | H | 10-25 | 50-75 | 100 | 75-100 | 50 |
| <i>Sst I</i> | L | 100 | 25-50 | 25 | <10 | 50 |
| <i>Sty I</i> | H | 25-50 | 75-100 | 100 | 75-100 | <10 |
| <i>Taq I</i> (65°C) | U | 10-25 | 50-75 | 75-100 | 50-75 | 50 |
| <i>Xba I</i> | M | 50-75 | 100 | 75 | 75 | 75 |

- Reactions were carried out at 37°C except for *Bcl I*, *BseA I*, *BseB I*, *BseC I*, *BsiS I*, *BssA I*, *BstE II*, *PspP I*, *Sfi I*, *Sma I* and *Taq I*. The reaction temperature for these enzymes is indicated in parenthesis.
- All reactions were carried out in the presence of BSA, 100µg/ml.

Suggested Buffers for Double Digestion

| | <i>Bam</i> HI | <i>Bgl</i> II | <i>Eco</i> RI | <i>Eco</i> RV | <i>Hind</i> III | <i>Kpn</i> I | <i>Nco</i> I | <i>Nhe</i> I | <i>Not</i> I | <i>Pst</i> I | <i>Pvu</i> II | <i>Sal</i> I | <i>Sgr</i> BI | <i>Sla</i> I | <i>Sma</i> I | <i>Sph</i> I | <i>Sst</i> I | |
|-----------------|----------------|---------------|---------------|---------------|-----------------|----------------|----------------|--------------|--------------|--------------|---------------|--------------|---------------|--------------|--------------|--------------|--------------|---|
| | U | H | U | M | M | U | H ⁺ | A | U | U | M | SH | L | SH | A | M | L | |
| <i>Bgl</i> II | H | H | | | | | | | | | | | | | | | | |
| <i>Eco</i> RI | U | | <i>Eco</i> RI | <i>Eco</i> RI | | | | | | | | | | | | | | |
| <i>Eco</i> RV | M | M | M | <i>Eco</i> RI | | | | | | | | | | | | | | |
| <i>Hind</i> III | M | M | M | <i>Eco</i> RI | M | | | | | | | | | | | | | |
| <i>Kpn</i> I | U | seq | M | seq | M | <i>Kpn</i> I/M | | | | | | | | | | | | |
| <i>Nco</i> I | H ⁺ | <i>Bam</i> HI | H | <i>Eco</i> RI | M | M | L | | | | | | | | | | | |
| <i>Nhe</i> I | A | A | M | A | A | M | L | A | | | | | | | | | | |
| <i>Not</i> I | U | <i>Bam</i> HI | H | <i>Eco</i> RI | H | M | seq | SH | A | | | | | | | | | |
| <i>Pst</i> I | U | <i>Bam</i> HI | H | <i>Eco</i> RI | M/H | M | seq | H | A | H | | | | | | | | |
| <i>Pvu</i> II | M | <i>Bam</i> HI | H | <i>Eco</i> RI | M | M | A | H | M | H | H | | | | | | | |
| <i>Sal</i> I | SH | SH | SH | <i>Eco</i> RI | H | seq | seq | SH | seq | SH | SH | H | | | | | | |
| <i>Sgr</i> BI | L | L/M | M | M/H | M | M | L | M | L | seq | H | M | seq | | | | | |
| <i>Sla</i> I | SH | <i>Bam</i> HI | H | <i>Eco</i> RI | M | M | seq | SH | M | SH | H | H | SH | M/H | | | | |
| <i>Sma</i> I | A | A | seq | seq | A | A | A | A | A | A | A | A | seq | seq | seq | | | |
| <i>Sph</i> I | M | M | M | <i>Eco</i> RI | M | M | L | M | L | <i>Not</i> I | M | M | SH | L | M | A | | |
| <i>Sst</i> I | L | L | M | seq | M | M | L | L | L | A | A | A | seq | L | seq | A | L | |
| <i>Xba</i> I | M | M | M/H | <i>Eco</i> RI | M | M | L | M | A | <i>Not</i> I | H | M | SH | M | M/SH | A | M | L |

Notes:

• All the reactions were carried out in the presence of BSA (100µg/ml). Our experience indicates that it is important to use BSA in reaction mixtures in order to obtain successful digestions of DNA. The presence of BSA gives complete and reproducible cleavages for a range of DNA substrates. BSA stabilizes the enzymes when digestions are performed for more than one hour at 37°C, since many restriction endonucleases in reaction buffers without BSA can survive at this temperature for 10-20 minutes only or even less. Also, BSA binds metal ions, and other chemicals, which might be present in buffers or DNA preparations, thereby inactivating restriction endonucleases. The following enzymes can exhibit "star" activity: *Bam*H I, *Bcl* I, *Bse*B I, *Bss*A I, *Eco*R I, *Eco*R V, *Hind* III, *Hpa* I, *Kpn* I, *Nco* I, *Nru* I, *Pst* I, *Pvu* II, *Sal* I, *Sca* I, *Sna*B I, *Sph* I, *Ssp* I, *Xba* I.