

NEBuffer Performance Chart with Restriction Enzymes























For your convenience, New England Biolabs offers a simple 4 buffer system. A color-coded 10X NEBuffer is supplied with every restriction endonuclease, ensuring 100% activity. Over 170 restriction enzymes exhibit 100% activity in NEBuffer 4, resulting in increased efficiency, flexibility and ease of use especially when performing double digests.

To help select the best conditions for double digests, this chart shows the optimal (supplied) NEBuffer and approximate activity in the four standard NEBuffers for each enzyme. In addition, the table shows recommended reaction temperature, heat inactivation temperature, recommended diluent buffer and whether the enzyme is Time-Saver qualified (i.e., cleaves substrate in 5-15 minutes under recommended conditions). Note: The values listed in this table are approximate. They were obtained using each enzyme's specific unit assay substrate DNA.







































Please check out other technical reference information related to restriction enzymes:

[Double Digestion](#) | [Heat Inactivation](#) | [Activity at 37°C](#) | [Diluent Buffers](#) | [Time-Saver Enzymes](#) | [High Fidelity \(HF\) Restriction Enzymes](#)

[Chart Legend](#) | [Icon Descriptions](#)













































| Enzyme | Supplied NEBuffer | %Activity in NEBuffer | | | | | Heat Inac. | Incu. Temp. | Diluent |
|------------------|--|-----------------------|-----|-----|-----|-------|------------|-------------|---------|
| | | 1 | 2 | 3 | 4 | EcoRI | | | |
| AatII |  NEBuffer 4 | 0 | 50 | 50 | 100 | 25 | 65°C | 37°C | A |
| Acc65I |  NEBuffer 3 + BSA | 10 | 75 | 100 | 25 | 50 | 65°C | 37°C | A |
| AccI |  NEBuffer 4 | 50 | 50 | 10 | 100 | 5 | 80°C | 37°C | A |
| AcI |  NEBuffer 3 | 25 | 50 | 100 | 50 | 100 | 65°C | 37°C | A |
| AcII |  NEBuffer 4 + BSA | 10 | 10 | 0 | 100 | 5 | | 37°C | B |
| AcuI |  NEBuffer 4 + SAM | 50 | 100 | 50 | 100 | 100 | 65°C | 37°C | B |
| AfeI |  NEBuffer 4 | 25 | 50 | 25 | 100 | 100 | 65°C | 37°C | B |
| AflII |  NEBuffer 4 + BSA | 50 | 100 | 25 | 100 | 0 | 65°C | 37°C | A |
| AflIII |  NEBuffer 3 + BSA | 25 | 75 | 100 | 50 | 100 | 80°C | 37°C | B |
| AgeI |  NEBuffer 1 | 100 | 50 | 10 | 75 | 50 | 65°C | 37°C | C |
| AgeI-HF™ |  NEBuffer 4 + BSA | 100 | 50 | 10 | 100 | 25 | 65°C | 37°C | A |
| AgeI-HF™ RE-Mix® |  - | - | - | - | - | - | 65°C | 37°C | - |
| AhdI |  NEBuffer 4 + BSA | 25 | 75 | 0 | 100 | 10 | 65°C | 37°C | A |
| AleI |  NEBuffer 4 | 10 | 25 | 10 | 100 | 0 | 65°C | 37°C | A |
| AluI |  NEBuffer 4 | 100 | 100 | 75 | 100 | 100 | 65°C | 37°C | B |
| AlwI |  NEBuffer 4 | 50 | 100 | 10 | 100 | 5 | 65°C | 37°C | A |
| AlwNI |  NEBuffer 4 | 10 | 100 | 100 | 100 | 10 | 65°C | 37°C | A |
| ApaI |  NEBuffer 4 + BSA | 25 | 50 | 0 | 100 | 5 | 65°C | 25°C | A |
| ApaLI |  NEBuffer 4 + BSA | 100 | 100 | 10 | 100 | 5 | | 37°C | A |
| ApeKI |  NEBuffer 3 | 25 | 75 | 100 | 50 | 100 | | 75°C | B |
| ApoI |  NEBuffer 3 + BSA | 10 | 75 | 100 | 75 | 100 | 80°C | 50°C | A |
| AscI |  NEBuffer 4 | 0 | 10 | 10 | 100 | 5 | 65°C | 37°C | A |

| | | | | | | | | | | | |
|--------------|--|----------------------|-----|-----|-------------------|-----|-----|------|------|------|---|
| Ascl RE-Mix® | | - | - | - | - | - | - | - | 65°C | 37°C | - |
| Asel | | NEBuffer 3 | N/R | 75 | 100 ^{dd} | N/R | 50 | 65°C | 37°C | B | |
| AsiSI | | NEBuffer 4 + BSA | 50 | 100 | 100 | 100 | 0 | 80°C | 37°C | B | |
| Aval | | NEBuffer 4 | 10 | 75 | 10 | 100 | 50 | 80°C | 37°C | A | |
| Avall | | NEBuffer 4 | 50 | 75 | 10 | 100 | 50 | 65°C | 37°C | A | |
| AvrII | | NEBuffer 4 | 100 | 100 | 50 | 100 | 50 | 80°C | 37°C | B | |
| BaeGI | | NEBuffer 1 | 100 | 75 | 10 | 50 | 100 | 65°C | 37°C | A | |
| Bael | | NEBuffer 4 + BSA+SAM | 50 | 100 | 50 | 100 | 40 | 65°C | 25°C | A | |
| BamHI | | NEBuffer 3 + BSA | 75 | 100 | 100 ^{dd} | 100 | 100 | | 37°C | A | |
| BamHI-HF™ | | NEBuffer 4 | 100 | 50 | 10 | 100 | 25 | | 37°C | A | |
| BanI | | NEBuffer 4 + BSA | 50 | 100 | 50 | 100 | 25 | 65°C | 37°C | A | |
| BanII | | NEBuffer 4 | 100 | 10 | 50 | 100 | 100 | 80°C | 37°C | A | |
| BbsI | | NEBuffer 2 | 100 | 100 | 25 | 75 | 50 | 65°C | 37°C | B | |
| BbvCI | | NEBuffer 4 | 50 | 100 | 10 | 100 | 100 | 80°C | 37°C | A | |
| BbvI | | NEBuffer 2 | 100 | 100 | 25 | 75 | 100 | 65°C | 37°C | B | |
| BccI | | NEBuffer 1 + BSA | 100 | 50 | 10 | 50 | 15 | 65°C | 37°C | A | |
| BceAI | | NEBuffer 3 + BSA | 100 | 100 | 100 | 100 | 100 | 65°C | 37°C | A | |
| BcgI | | NEBuffer 3 + SAM | N/R | N/R | 100 ^{dd} | N/R | 50 | 65°C | 37°C | A | |
| BciVI | | NEBuffer 4 | 100 | 50 | 0 | 100 | 25 | 65°C | 37°C | C | |
| BclI | | NEBuffer 3 | 50 | 100 | 100 ^{dd} | 75 | 100 | | 50°C | A | |
| BcoDI | | NEBuffer 4 | 50 | 75 | 75 | 100 | 100 | 65°C | 37°C | B | |
| Bfal | | NEBuffer 4 | 75 | 50 | 10 | 100 | 0 | 80°C | 37°C | B | |
| BfuAI | | NEBuffer 3 | 0 | 75 | 100 | 10 | 100 | 65°C | 50°C | B | |
| BfuCI | | NEBuffer 4 + BSA | 100 | 50 | 25 | 100 | 15 | 80°C | 37°C | B | |
| BglI | | NEBuffer 3 | 50 | 75 | 100 | 50 | 100 | 65°C | 37°C | B | |
| BglII | | NEBuffer 3 | 10 | 75 | 100 | 10 | 100 | | 37°C | A | |
| BlnI | | NEBuffer 4 | 50 | 100 | 10 | 100 | 50 | | 37°C | A | |
| BmgBI | | NEBuffer 3 + BSA | 0 | 25 | 100 | 10 | 50 | 65°C | 37°C | B | |
| Bmrl | | NEBuffer 2 | 75 | 100 | 75 | 100 | 50 | 65°C | 37°C | B | |
| BmtI | | NEBuffer 2 | 25 | 100 | 25 | 50 | 100 | 65°C | 37°C | B | |
| BmtI-HF™ | | NEBuffer 4 + BSA | 50 | 100 | 10 | 100 | N/R | 65°C | 37°C | B | |
| Bpml | | NEBuffer 3 + BSA | 75 | 100 | 100 | 100 | 100 | 65°C | 37°C | B | |
| Bpu10I | | NEBuffer 3 | 10 | 25 | 100 | 25 | 100 | 80°C | 37°C | B | |
| BpuEI | | NEBuffer 4 + SAM | 50 | 100 | 10 | 100 | 100 | 65°C | 37°C | B | |
| BsaAI | | NEBuffer 4 | 100 | 100 | 100 | 100 | 100 | | 37°C | C | |
| BsaBI | | NEBuffer 4 | 50 | 100 | 75 | 100 | 100 | 80°C | 60°C | B | |
| BsaHI | | NEBuffer 4 + BSA | 50 | 100 | 100 | 100 | 100 | 80°C | 37°C | A | |
| Bsal | | NEBuffer 4 + BSA | 75 | 75 | 100 | 100 | 100 | 65°C | 37°C | B | |

| | | | | | | | | | | |
|--------------------|---|----------------------|-----|-----|-------------------|-----|-----|------|------|---|
| Bsal-HF™ |  | NEBuffer 4 + BSA | 25 | 25 | 10 | 100 | 10 | 65°C | 37°C | B |
| BsaJI |  | NEBuffer 4 | 100 | 100 | 100 | 100 | 100 | 80°C | 60°C | A |
| BsaWI |  | NEBuffer 4 + BSA | 50 | 100 | 50 | 100 | 40 | 80°C | 60°C | A |
| BsaXI |  | NEBuffer 4 | 75 | 100 | 10 | 100 | 15 | | 37°C | B |
| BseRI |  | NEBuffer 4 | 100 | 100 | 75 | 100 | 100 | 65°C | 37°C | A |
| BseYI |  | NEBuffer 3 | 10 | 50 | 100 | 50 | 100 | 65°C | 37°C | B |
| BsgI |  | NEBuffer 4 + SAM | 50 | 75 | 50 | 100 | 20 | 65°C | 37°C | B |
| BsiEI |  | NEBuffer 4 + BSA | 50 | 100 | 10 | 100 | 5 | 80°C | 60°C | A |
| BsiHKA1 | | NEBuffer 4 + BSA | 50 | 100 | 100 | 100 | 25 | | 65°C | B |
| BsiWI |  | NEBuffer 3 | 100 | 100 | 100 | 25 | 100 | 80°C | 55°C | A |
| BsII |  | NEBuffer 3 | 10 | 50 | 100 | 75 | 100 | 80°C | 55°C | A |
| BsmAI |  | NEBuffer 4 | 50 | 100 | 100 | 100 | 100 | 80°C | 55°C | B |
| BsmBI |  | NEBuffer 3 | 75 | 100 | 100 | 100 | 100 | 80°C | 55°C | B |
| BsmFI |  | NEBuffer 4 + BSA | 25 | 50 | 50 | 100 | 15 | 80°C | 65°C | A |
| BsmI |  | NEBuffer 4 | 75 | 100 | 75 | 100 | 5 | 80°C | 65°C | A |
| BsoBI |  | NEBuffer 2 | 10 | 100 | 100 | 50 | 100 | | 37°C | A |
| Bsp1286I |  | NEBuffer 4 + BSA | 25 | 25 | 25 | 100 | 10 | 65°C | 37°C | A |
| BspCNI |  | NEBuffer 4 + BSA+SAM | 100 | 75 | 10 | 100 | 15 | 80°C | 25°C | A |
| BspDI |  | NEBuffer 4 | 25 | 75 | 50 | 100 | 100 | 65°C | 37°C | A |
| BspEI |  | NEBuffer 3 | 0 | 10 | 100 | 0 | N/R | 80°C | 37°C | B |
| BspHI |  | NEBuffer 4 | 10 | 100 | 50 | 100 | 5 | 65°C | 37°C | A |
| BspMI |  | NEBuffer 3 | N/R | N/R | 100 | N/R | 100 | 65°C | 37°C | B |
| BspQI |  | NEBuffer 4 | 10 | 50 | 100 | 100 | 100 | 80°C | 50°C | B |
| BsrBI |  | NEBuffer 4 | 50 | 100 | 100 | 100 | 100 | 80°C | 37°C | A |
| BsrDI |  | NEBuffer 2 + BSA | 50 | 100 | 50 | 75 | 50 | 80°C | 65°C | A |
| BsrFI |  | NEBuffer 4 | 10 | 100 | 100 | 100 | 100 | | 37°C | C |
| BsrGI |  | NEBuffer 2 + BSA | 25 | 100 | 10 | 100 | 50 | 80°C | 37°C | A |
| BsrI |  | NEBuffer 3 | 0 | 50 | 100 | 10 | 100 | 80°C | 65°C | B |
| BssHII |  | NEBuffer 3 | 100 | 100 | 100 ^{dd} | 100 | 100 | 80°C | 50°C | B |
| BssKI |  | NEBuffer 3 + BSA | 0 | 50 | 100 | 50 | 100 | 80°C | 60°C | A |
| BssSI |  | NEBuffer 3 + BSA | 0 | 50 | 100 ^{dd} | 10 | 100 | 80°C | 37°C | B |
| BstAPI |  | NEBuffer 4 + BSA | 25 | 100 | 100 | 100 | 100 | 80°C | 60°C | A |
| BstBI |  | NEBuffer 4 | 75 | 50 | 25 | 100 | 50 | | 65°C | A |
| BstEII |  | NEBuffer 3 + BSA | 50 | 75 | 100 | 75 | 100 | | 60°C | A |
| BstEII-HF™ |  | NEBuffer 4 + BSA | 50 | 75 | 100 | 100 | N/R | | 37°C | A |
| BstEII-HF™ RE-Mix® |  | - | - | - | - | - | - | | 37°C | - |
| BstNI |  | NEBuffer 2 + BSA | 10 | 100 | 100 | 75 | 50 | | 60°C | A |
| BstUI |  | NEBuffer 4 | 100 | 100 | 50 | 100 | 100 | | 60°C | A |
| BstXI |  | NEBuffer 3 | 0 | 50 | 100 | 25 | 100 | 65°C | 37°C | B |






| | | | | | | | | | | |
|-------------------|--|----------------------|-----|-----|-------------------|-----|-----|------|------|---|
| BstYI | | NEBuffer 2 | 50 | 100 | 75 | 100 | 50 | 80°C | 60°C | A |
| BstZ17I | | NEBuffer 4 | N/R | N/R | 100 | 100 | 100 | | 37°C | B |
| Bsu36I | | NEBuffer 3 + BSA | 0 | 25 | 100 | 0 | 15 | 80°C | 37°C | A |
| BtgI | | NEBuffer 3 + BSA | 25 | 50 | 100 | 100 | 100 | 80°C | 37°C | B |
| BtgZI | | NEBuffer 4 + BSA | 10 | 25 | 0 | 100 | 50 | 80°C | 60°C | A |
| BtsCI | | NEBuffer 4 | 50 | 100 | 50 | 100 | 25 | | 50°C | B |
| BtsI | | NEBuffer 4 + BSA | 100 | 50 | 50 | 100 | 25 | 80°C | 55°C | A |
| BtsIMutI | | NEBuffer 4 + BSA | 100 | 50 | 10 | 100 | N/R | 80°C | 55°C | A |
| Cac8I | | NEBuffer 4 | 50 | 75 | 100 | 100 | 50 | 65°C | 37°C | B |
| Clal | | NEBuffer 4 + BSA | 10 | 50 | 50 | 100 | 50 | 65°C | 37°C | A |
| CspCI | | NEBuffer 4 + SAM | 10 | 100 | 10 | 100 | 20 | 65°C | 37°C | A |
| CviAII | | NEBuffer 4 + BSA | 75 | 25 | 10 | 100 | 25 | 65°C | 25°C | C |
| CviKI-1 | | NEBuffer 4 | 10 | 50 | 25 | 100 | 100 | 80°C | 37°C | A |
| CviQI | | NEBuffer 3 + BSA | 75 | 100 | 100 | 75 | 0 | | 25°C | C |
| Ddel | | NEBuffer 3 | 75 | 100 | 100 | 75 | 100 | 65°C | 37°C | A |
| Dpnl | | NEBuffer 4 | 100 | 100 | 75 | 100 | 100 | 80°C | 37°C | B |
| DpnII | | NEBuffer DpnII | N/R | N/R | 100 ^{dd} | N/R | 100 | 65°C | 37°C | B |
| DraI | | CutSmart Buffer | N/R | N/R | N/R | N/R | N/R | 65°C | 37°C | A |
| DraIII | | NEBuffer 3 + BSA | 100 | 75 | 100 | 25 | 50 | 65°C | 37°C | B |
| DraIII-HF™ | | NEBuffer 4 + BSA | 0 | 50 | 10 | 100 | 50 | | 37°C | B |
| DrdI | | NEBuffer 4 + BSA | 25 | 50 | 10 | 100 | 100 | 65°C | 37°C | A |
| EaeI | | NEBuffer 4 | 100 | 100 | 50 | 100 | 100 | 80°C | 37°C | A |
| EagI | | NEBuffer 3 | 10 | 25 | 100 | 10 | 100 | 65°C | 37°C | C |
| EagI-HF™ | | NEBuffer 4 | 25 | 100 | 100 | 100 | 100 | 65°C | 37°C | B |
| EarI | | NEBuffer 4 | 100 | 100 | 50 | 100 | 100 | 65°C | 37°C | A |
| Ecil | | NEBuffer 4 | 100 | 50 | 50 | 100 | N/R | 65°C | 37°C | A |
| Eco53kl | | NEBuffer 4 | 100 | 50 | 25 | 100 | 10 | 65°C | 37°C | A |
| EcoNI | | NEBuffer 4 | 100 | 100 | 75 | 100 | 100 | 65°C | 37°C | A |
| EcoO109I | | NEBuffer 4 + BSA | 100 | 100 | 75 | 100 | 15 | 65°C | 37°C | A |
| EcoP15I | | NEBuffer 3 + BSA+ATP | 75 | 100 | 100 | 100 | 50 | 65°C | 37°C | A |
| EcoRI | | NEBuffer EcoRI | 100 | 100 | 100 | 100 | 100 | 65°C | 37°C | C |
| EcoRI-HF™ | | NEBuffer 4 | 10 | 100 | 0 | 100 | 5 | 65°C | 37°C | C |
| EcoRI-HF™ RE-Mix® | | - | - | - | - | - | - | 65°C | 37°C | - |
| EcoRV | | NEBuffer 3 + BSA | 50 | 75 | 100 | 50 | 50 | 80°C | 37°C | A |
| EcoRV-HF™ | | NEBuffer 4 | 25 | 100 | 100 | 100 | 100 | 65°C | 37°C | B |
| EcoRV-HF™ RE-Mix® | | - | - | - | - | - | - | 65°C | 37°C | - |
| FatI | | NEBuffer 2 | 10 | 100 | 50 | 50 | 100 | 65°C | 55°C | A |
| FauI | | NEBuffer 4 | 100 | 50 | 10 | 100 | 50 | 65°C | 55°C | A |

| | | | | | | | | | | |
|------------------|--|-----------------------|-----|-----|-----|-----|-----|------|------|---|
| Fnu4HI | | NEBuffer 4 | 10 | 25 | 25 | 100 | 10 | 65°C | 37°C | A |
| FokI | | NEBuffer 4 | 100 | 100 | 75 | 100 | 25 | 65°C | 37°C | A |
| FseI | | NEBuffer 4 + BSA | 100 | 75 | 0 | 100 | 50 | 65°C | 37°C | B |
| FspEI | | NEBuffer 4 + BSA | N/R | N/R | N/R | 100 | N/R | 65°C | 37°C | B |
| FspI | | NEBuffer 4 | 10 | 75 | 10 | 100 | 50 | 65°C | 37°C | C |
| HaeII | | NEBuffer 4 + BSA | 75 | 100 | 50 | 100 | 25 | 80°C | 37°C | A |
| HaeIII | | NEBuffer 4 | 50 | 100 | 25 | 100 | 100 | 80°C | 37°C | A |
| HgaI | | NEBuffer 1 | 100 | 75 | 50 | 100 | 25 | 65°C | 37°C | A |
| HhaI | | NEBuffer 4 + BSA | 75 | 100 | 100 | 100 | 100 | 65°C | 37°C | A |
| HincII | | NEBuffer 3 + BSA | 75 | 100 | 100 | 100 | 100 | 65°C | 37°C | B |
| HindIII | | NEBuffer 2 | 50 | 100 | 10 | 50 | 25 | 65°C | 37°C | B |
| HindIII-HF™ | | NEBuffer 4 | 10 | 100 | 10 | 100 | 10 | 80°C | 37°C | B |
| Hinfl | | NEBuffer 4 | 75 | 100 | 75 | 100 | 100 | 80°C | 37°C | A |
| HinP1I | | NEBuffer 4 | 100 | 100 | 100 | 100 | 100 | 65°C | 37°C | A |
| HpaI | | NEBuffer 4 | 25 | 25 | 10 | 100 | 100 | | 37°C | A |
| HpaII | | NEBuffer 1 | 100 | 50 | 10 | 50 | 15 | 65°C | 37°C | A |
| HphI | | NEBuffer 4 | N/R | 75 | 0 | 100 | 10 | 65°C | 37°C | B |
| Hpy166II | | NEBuffer 4 | 100 | 100 | 50 | 100 | 20 | 65°C | 37°C | C |
| Hpy188I | | NEBuffer 4 | 50 | 25 | 10 | 100 | 50 | 65°C | 37°C | A |
| Hpy188III | | NEBuffer 4 + BSA | 100 | 100 | 10 | 100 | 5 | 65°C | 37°C | A |
| Hpy99I | | NEBuffer 4 + BSA | 10 | 10 | N/R | 100 | 25 | 65°C | 37°C | A |
| HpyAV | | NEBuffer 4 + BSA | 100 | 100 | 25 | 100 | 50 | 65°C | 37°C | B |
| HpyCH4III | | NEBuffer 4 | 100 | 50 | 25 | 100 | 20 | 80°C | 37°C | A |
| HpyCH4IV | | NEBuffer 1 | 100 | 25 | 10 | 25 | 15 | 65°C | 37°C | A |
| HpyCH4V | | NEBuffer 4 | 50 | 50 | 25 | 100 | 50 | 65°C | 37°C | A |
| I-CeuI | | NEBuffer 4 + BSA | 10 | 10 | 0 | 100 | N/R | 65°C | 37°C | B |
| I-SceI | | NEBuffer I-SceI + BSA | 10 | 50 | 50 | 50 | N/R | 65°C | 37°C | B |
| KasI | | NEBuffer 4 + BSA | 25 | 100 | 0 | 100 | 100 | 65°C | 37°C | B |
| KpnI | | NEBuffer 1 + BSA | 100 | 75 | 0 | 50 | 25 | | 37°C | A |
| KpnI-HF™ | | NEBuffer 4 | 100 | 25 | 0 | 100 | 0 | | 37°C | A |
| KpnI-HF™ RE-Mix® | | - | - | - | - | - | - | | 37°C | - |
| LpnPI | | NEBuffer 4 + BSA | N/R | N/R | N/R | 100 | N/R | 65°C | 37°C | B |
| MboI | | NEBuffer 4 | 75 | 100 | 100 | 100 | 100 | 65°C | 37°C | A |
| MboII | | NEBuffer 4 | 100 | 100 | 50 | 100 | 100 | 65°C | 37°C | C |
| MfeI | | NEBuffer 4 | 75 | 50 | 10 | 100 | 5 | 65°C | 37°C | A |
| MfeI-HF™ | | NEBuffer 4 | 75 | 50 | 10 | 100 | 0 | 65°C | 37°C | A |
| MfeI-HF™ RE-Mix® | | - | - | - | - | - | - | 65°C | 37°C | - |
| MluCI | | NEBuffer 4 | 100 | 10 | 10 | 100 | 0 | 80°C | 37°C | A |
| MluI | | NEBuffer 3 | 25 | 75 | 100 | 50 | 100 | 65°C | 37°C | A |

| | | | | | | | | | | |
|------------------|---|------------------|-----|-----|-----|-------------------|-----|------|------|---|
| MlyI |  | NEBuffer 4 + BSA | 50 | 50 | 25 | 100 | 25 | 65°C | 37°C | A |
| MmeI |  | NEBuffer 4 + SAM | N/R | N/R | N/R | 100 ^{dd} | 50 | 80°C | 37°C | B |
| MnlI |  | NEBuffer 4 + BSA | 75 | 100 | 50 | 100 | 50 | 65°C | 37°C | B |
| MscI |  | NEBuffer 4 | 75 | 75 | 75 | 100 | 100 | 65°C | 37°C | B |
| MseI |  | NEBuffer 4 + BSA | 75 | 100 | 75 | 100 | 50 | 65°C | 37°C | A |
| MslI |  | NEBuffer 4 | 100 | 100 | 25 | 100 | 10 | 65°C | 37°C | A |
| MspA1I |  | NEBuffer 4 + BSA | 50 | 100 | 50 | 100 | 50 | 65°C | 37°C | B |
| Mspl |  | NEBuffer 4 | 75 | 100 | 50 | 100 | 50 | | 37°C | A |
| MspJI |  | NEBuffer 4 + BSA | N/R | N/R | N/R | 100 | N/R | 65°C | 37°C | B |
| Mwol |  | NEBuffer 3 | 10 | 75 | 100 | 75 | 100 | | 60°C | B |
| NaeI |  | NEBuffer 4 | 100 | 75 | 10 | 100 | 25 | 65°C | 37°C | A |
| NarI |  | NEBuffer 4 | 100 | 75 | 75 | 100 | 50 | 65°C | 37°C | A |
| Nb.BbvCI |  | NEBuffer 2 | 50 | 100 | 10 | 100 | N/R | 80°C | 37°C | A |
| Nb.BsmI |  | NEBuffer 3 | 25 | 100 | 100 | 25 | N/R | 80°C | 65°C | A |
| Nb.BsrDI |  | NEBuffer 2 | 10 | 100 | 25 | 50 | N/R | 80°C | 65°C | A |
| Nb.BtsI |  | NEBuffer 4 + BSA | 75 | 100 | 75 | 100 | N/R | 80°C | 37°C | A |
| NciI |  | NEBuffer 4 | 100 | 25 | 10 | 100 | 25 | | 37°C | A |
| NcoI |  | NEBuffer 3 | 100 | 100 | 100 | 100 | 100 | 65°C | 37°C | A |
| NcoI-HF™ |   | NEBuffer 4 | 50 | 100 | 10 | 100 | 100 | 80°C | 37°C | B |
| NcoI-HF™ RE-Mix® |   | - | - | - | - | - | - | 80°C | 37°C | - |
| NdeI |  | NEBuffer 4 | 75 | 100 | 75 | 100 | 100 | 65°C | 37°C | A |
| NgoMIV |  | NEBuffer 4 | 100 | 50 | 10 | 100 | 0 | 80°C | 37°C | A |
| NheI |  | NEBuffer 2 + BSA | 100 | 100 | 10 | 100 | 15 | 65°C | 37°C | C |
| NheI-HF™ |   | NEBuffer 4 + BSA | 100 | 10 | 0 | 100 | 0 | 80°C | 37°C | C |
| NheI-HF™ RE-Mix® |   | - | - | - | - | - | - | 80°C | 37°C | - |
| NlaIII |  | NEBuffer 4 + BSA | 25 | 25 | 25 | 100 | 5 | 65°C | 37°C | B |
| NlaIV |  | NEBuffer 4 + BSA | 10 | 10 | 10 | 100 | 100 | 65°C | 37°C | B |
| NmeAIII |  | NEBuffer 4 + SAM | 10 | 10 | 0 | 100 | 0 | 80°C | 37°C | B |
| NotI |  | NEBuffer 3 + BSA | 0 | 50 | 100 | 25 | 100 | 65°C | 37°C | C |
| NotI-HF™ |   | NEBuffer 4 + BSA | 25 | 100 | 25 | 100 | 50 | 65°C | 37°C | A |
| NotI-HF™ RE-Mix® |   | - | - | - | - | - | - | 65°C | 37°C | - |
| NruI |  | NEBuffer 3 | 0 | 10 | 100 | 10 | 100 | 65°C | 37°C | A |
| NsiI |  | NEBuffer 3 | 10 | 75 | 100 | 25 | 100 | 80°C | 37°C | A |
| NspI |  | NEBuffer 2 + BSA | 100 | 100 | 0 | 100 | 5 | 65°C | 37°C | A |
| Nt.AlwI |  | NEBuffer 2 | 25 | 100 | 25 | 50 | N/R | 80°C | 37°C | A |
| Nt.BbvCI |  | NEBuffer 4 | 50 | 100 | 10 | 100 | N/R | 80°C | 37°C | A |
| Nt.BsmAI |  | NEBuffer 4 | 100 | 50 | 10 | 100 | N/R | 65°C | 37°C | A |
| Nt.BspQI |  | NEBuffer 3 | 10 | 75 | 100 | 25 | N/R | 80°C | 50°C | B |

| | | | | | | | | | | |
|------------------|--|------------------------|-----|-----|-----|-----|-----|------|------|---|
| Nt.BstNBI | | NEBuffer 3 | 0 | 10 | 100 | 10 | N/R | 80°C | 55°C | A |
| Nt.CviPII | | NEBuffer 4 | 25 | 100 | 50 | 100 | N/R | 65°C | 37°C | A |
| Pacl | | NEBuffer 1 + BSA | 100 | 75 | 10 | 100 | 5 | 65°C | 37°C | A |
| Pacl RE-Mix® | | - | - | - | - | - | - | 65°C | 37°C | - |
| PaeR7I | | NEBuffer 4 | 25 | 100 | 10 | 100 | 50 | | 37°C | A |
| Pcil | | NEBuffer 3 + BSA | 50 | 75 | 100 | 50 | N/R | 80°C | 37°C | B |
| PflFI | | NEBuffer 4 + BSA | 0 | 25 | 25 | 100 | 0 | 65°C | 37°C | A |
| PflMI | | NEBuffer 3 + BSA | 0 | 100 | 100 | 50 | 100 | 65°C | 37°C | A |
| Phol | | NEBuffer 3 | 50 | 50 | 100 | 75 | 100 | | 75°C | A |
| PI-PspI | | NEBuffer PI-PspI + BSA | 0 | 10 | 10 | 10 | N/R | | 65°C | B |
| PI-SceI | | NEBuffer PI-SceI + BSA | 0 | 0 | 0 | 0 | N/R | 65°C | 37°C | B |
| PleI | | NEBuffer 4 | 10 | 100 | 100 | 100 | 50 | 65°C | 37°C | A |
| PluTI | | NEBuffer 4 | 100 | 25 | 5 | 100 | N/R | 65°C | 37°C | A |
| Pmel | | NEBuffer 4 + BSA | 0 | 50 | 10 | 100 | 5 | 65°C | 37°C | A |
| PmlI | | NEBuffer 1 + BSA | 100 | 75 | 0 | 75 | 15 | 65°C | 37°C | A |
| PpuMI | | NEBuffer 4 | 0 | 25 | 0 | 100 | 10 | | 37°C | B |
| PshAI | | NEBuffer 4 + BSA | 50 | 50 | 0 | 100 | 5 | 65°C | 37°C | A |
| Psil | | NEBuffer 4 | 10 | 100 | 10 | 100 | 10 | 65°C | 37°C | B |
| PspGI | | NEBuffer 4 | 75 | 100 | 50 | 100 | 100 | | 75°C | A |
| PspOMI | | NEBuffer 4 | 25 | 25 | 10 | 100 | 15 | 65°C | 37°C | B |
| PspXI | | NEBuffer 4 | 0 | 100 | 10 | 100 | 50 | 80°C | 37°C | B |
| PstI | | NEBuffer 3 + BSA | 75 | 75 | 100 | 50 | 50 | 80°C | 37°C | C |
| PstI-HF™ | | NEBuffer 4 | 10 | 75 | 50 | 100 | 25 | 80°C | 37°C | C |
| PvuI | | NEBuffer 3 + BSA | 10 | 75 | 100 | 10 | 100 | | 37°C | B |
| PvuI-HF™ | | NEBuffer 4 + BSA | 25 | 100 | 100 | 100 | 100 | | 37°C | B |
| PvuII | | NEBuffer 2 | 100 | 100 | 100 | 100 | 100 | | 37°C | B |
| PvuII-HF™ | | NEBuffer 4 | 0 | 25 | 0 | 100 | 0 | 80°C | 37°C | B |
| RsaI | | NEBuffer 4 | 100 | 100 | 50 | 100 | 15 | 65°C | 37°C | A |
| RsrII | | NEBuffer 4 | 25 | 75 | 10 | 100 | 25 | 65°C | 37°C | C |
| SacI | | NEBuffer 1 + BSA | 100 | 50 | 10 | 100 | 15 | 65°C | 37°C | A |
| SacI-HF™ | | NEBuffer 4 + BSA | 100 | 50 | 10 | 100 | 15 | 65°C | 37°C | A |
| SacII | | NEBuffer 4 | 25 | 75 | 10 | 100 | 100 | 65°C | 37°C | A |
| Sall | | NEBuffer 3 + BSA | 0 | 0 | 100 | 0 | 50 | 65°C | 37°C | A |
| Sall-HF™ | | NEBuffer 4 | 10 | 100 | 100 | 100 | 100 | 65°C | 37°C | A |
| Sall-HF™ RE-Mix® | | - | - | - | - | - | - | 80°C | 37°C | - |
| SapI | | NEBuffer 4 | 75 | 50 | 0 | 100 | 50 | 65°C | 37°C | B |
| Sau3AI | | NEBuffer 1 + BSA | 100 | 50 | 10 | 100 | 5 | 65°C | 37°C | A |
| Sau96I | | NEBuffer 4 | 50 | 100 | 100 | 100 | 50 | 80°C | 37°C | A |
| SbfI | | NEBuffer 4 | 75 | 50 | 0 | 100 | 25 | 65°C | 37°C | A |

| | | | | | | | | | | |
|--------------------|--|------------------|-----|-----|-------------------|-------------------|-----|------|------|---|
| Sbfl-HF™ | | NEBuffer 4 | 50 | 25 | 0 | 100 | 0 | 65°C | 37°C | B |
| Scal | | NEBuffer 3 | N/R | N/R | 100 ^{dd} | N/R | N/R | 80°C | 37°C | A |
| Scal-HF™ | | NEBuffer 4 | 100 | 100 | 10 | 100 | 15 | 65°C | 37°C | B |
| Scal-HF™ RE-Mix® | | - | - | - | - | - | - | 65°C | 37°C | - |
| ScrFl | | NEBuffer 4 | 100 | 100 | 100 | 100 | 100 | 65°C | 37°C | C |
| SexAI | | NEBuffer 4 + BSA | 100 | 75 | 50 | 100 | 50 | 65°C | 37°C | A |
| SfaNI | | NEBuffer 3 | 0 | 75 | 100 | 25 | 50 | 65°C | 37°C | B |
| Sfcl | | NEBuffer 4 + BSA | 75 | 50 | 10 | 100 | 25 | 65°C | 37°C | B |
| Sfil | | NEBuffer 4 + BSA | 0 | 100 | 10 | 100 | 15 | | 50°C | C |
| Sfol | | NEBuffer 4 | 25 | 100 | 50 | 100 | 100 | | 37°C | B |
| SgrAI | | NEBuffer 4 | 100 | 50 | 10 | 100 | 50 | 65°C | 37°C | A |
| Smal | | NEBuffer 4 | 0 | 0 | 0 | 100 | N/R | 65°C | 25°C | A |
| SmlI | | NEBuffer 4 + BSA | 25 | 75 | 25 | 100 | 50 | | 55°C | A |
| SnaBI | | NEBuffer 4 + BSA | 25 | 50 | 25 | 100 ^{dd} | 5 | 80°C | 37°C | A |
| Spel | | NEBuffer 4 + BSA | 75 | 100 | 25 | 100 | 50 | 80°C | 37°C | C |
| Spel RE-Mix® | | - | - | - | - | - | - | 80°C | 37°C | - |
| Spel-HF™ | | NEBuffer 4 + BSA | 25 | 50 | 0 | 100 | N/R | 80°C | 37°C | C |
| SphI-HF™ | | NEBuffer 4 | 50 | 25 | 10 | 100 | 0 | 65°C | 37°C | B |
| SphI | | NEBuffer 2 | 100 | 100 | 50 | 100 | 50 | 65°C | 37°C | B |
| Sspl | | NEBuffer Sspl | 50 | 100 | 50 | 50 | 100 | 65°C | 37°C | C |
| Sspl-HF™ | | NEBuffer 4 | 25 | 100 | 0 | 100 | 25 | 65°C | 37°C | B |
| StuI | | NEBuffer 4 | 100 | 100 | 50 | 100 | 50 | 65°C | 37°C | A |
| StyD4I | | NEBuffer 2 | 10 | 100 | 100 | 100 | 20 | 65°C | 37°C | B |
| StyI | | NEBuffer 3 + BSA | 25 | 75 | 100 | 10 | 15 | 65°C | 37°C | A |
| StyI-HF™ | | NEBuffer 4 | 25 | 100 | 25 | 100 | 0 | 65°C | 37°C | A |
| Swal | | NEBuffer 3 + BSA | 10 | 10 | 100 | 10 | 15 | 65°C | 25°C | B |
| Taq ^q I | | NEBuffer 4 + BSA | 50 | 75 | 100 | 100 | 100 | 80°C | 65°C | B |
| TfiI | | NEBuffer 4 | 100 | 100 | 100 | 100 | 100 | | 65°C | C |
| TliI | | NEBuffer 3 + BSA | 25 | 25 | 100 | 10 | 50 | | 75°C | B |
| Tsel | | NEBuffer 4 | 75 | 100 | 100 | 100 | 100 | | 65°C | B |
| Tsp45I | | NEBuffer 1 + BSA | 100 | 25 | 0 | 75 | 50 | | 65°C | A |
| Tsp509I | | NEBuffer 1 | 100 | 100 | 100 | N/R | 100 | | 65°C | A |
| TspMI | | NEBuffer 4 | 50 | 75 | 50 | 100 | 20 | | 75°C | B |
| TspRI | | NEBuffer 4 + BSA | 25 | 50 | 25 | 100 | 5 | | 65°C | B |
| Tth111I | | NEBuffer 4 | 50 | 25 | 25 | 100 | 100 | | 65°C | B |
| XbaI | | NEBuffer 4 + BSA | 0 | 100 | 75 | 100 | 15 | 65°C | 37°C | A |
| XbaI RE-Mix® | | - | - | - | - | - | - | 65°C | 37°C | - |
| XcmI | | NEBuffer 2 | 10 | 100 | 50 | 50 | 20 | 65°C | 37°C | C |

| | | | | | | | | | | |
|--------------|---|------------------|-----|-----|-----|-----|-----|------|------|---|
| XhoI |  | NEBuffer 4 + BSA | 75 | 100 | 100 | 100 | 100 | 65°C | 37°C | A |
| XhoI RE-Mix® |  | - | - | - | - | - | - | 80°C | 37°C | - |
| XmaI |  | NEBuffer 4 + BSA | 25 | 50 | 0 | 100 | 25 | 65°C | 37°C | A |
| XmnI |  | NEBuffer 4 + BSA | 100 | 100 | 50 | 100 | 5 | 65°C | 37°C | A |
| ZraI |  | NEBuffer 4 | 100 | 25 | 10 | 100 | 10 | | 37°C | B |

Note:

1. SspI Unique Buffer has the same composition as EcoRI Unique Buffer.
2. The values listed in this table are approximate. They were obtained using each enzyme's specific unit assay substrate DNA.