

Recommended Universal Buffer for Double Digestion

Double digestion (cutting a DNA with two restriction enzymes simultaneously) is used widely to save time. Takara provides universal buffers and the relative activity in each reaction buffer is shown on the table "Restriction enzyme activity displaying system using universal and basal buffers." In some combinations, however, it may be difficult to select suitable buffer for double digestion. In this table, the suitable buffer type, dilution rate and additive are shown to perform double digestion with enzymes that have a cutting site in a cloning site of pUC vectors. The characters,

such as L, M, H, T and K, show the type of buffer, and figures in "figure x" show the final concentration of each buffer. We supply universal buffers at 10-fold (10X) concentration. When "0.5X" is recommended, dilute 10X buffer by 20-fold, "1X" is recommended, dilute by 10-fold, and "2X" is by 5-fold for the use in double digestion. As BSA is also supplied at 10x concentration, dilute it by 10-fold to a final concentration of 0.1% when it is used.

| Enzyme | Acc I | BamH I | Bgl II | Cla I | EcoR I | EcoR V | Hinc II | Hind III | Kpn I | Nco I | Nde I | Not I | Pst I | Pvu I | Sac I | Sal I | Sma I | Spe I | Sph I | Xba I | Xho I |
|------------------------|---------------|---------------|-------------|-------------|-------------|---------------|---------------|---------------|---------------|---------------|-------------|-----------------------|---------------|---------------|---------------|---------------|---------------|-------------|---------------|---------------|-------------|
| Supplied Buffer | 10 × M | 10 × K | 10 × H | 10 × M | 10 × H | 10 × H | 10 × M | 10 × M | 10 × L | 10 × K + BSA | 10 × H | 10 × H + BSA + Triton | 10 × H | 10 × K + BSA | 10 × L | 10 × H | 10 × T + BSA | 10 × M | 10 × H | 10 × M + BSA | 10 × H |
| Acc I | — | 0.5 × K | 1 × T | 1 × M | 1 × M | 0.5 × K | 1 × M | 1 × M | 1 × M | 1 × M + BSA | 1 × T | 0.5 × K + BSA | 1 × M | 0.5 × K | 1 × M | 1.5 × T | 1 × T + BSA | 1 × M | 0.5 × K | 1 × M | 1 × M |
| BamH I | 0.5 × K | — | 1 × K | 1 × K | 1 × K | 1 × K | 0.5 × K | 1 × K | 0.5 × K | 1 × K + BSA | 1 × K | 0.5 × K + BSA | 1 × K | 1 × K | 0.5 × K | 1.5 × T | 0.5 × T + BSA | 1 × K | 1 × K | 0.5 × K | 1 × K |
| Bgl II | 1 × T | 1 × K | — | 1 × H | 1 × H | 1 × H | 2 × K | 1 × K | 1 × T | 1 × K + BSA | 1 × H | 1 × H + BSA | 1 × H | 1 × K | 0.5 × K | 1 × H | 1 × T + BSA | 1 × H | 1 × H | 2 × T | 1 × H |
| Cla I | 1 × M | 1 × K | 1 × H | — | 1 × H | 1 × H | 1 × M | 1 × M | 1 × M | 1 × K + BSA | 1 × H | 1 × H + BSA | 1 × H | 1 × K | 1 × M | 1 × H | 1 × T + BSA | 1 × M | 1 × H | 1 × M | 1 × H |
| EcoR I | 1 × M | 1 × K | 1 × H | 1 × H | — | 1 × H | 1 × M | 1 × M | 1 × M | 1 × K + BSA | 1 × H | 1 × H + BSA | 1 × H | 1 × K | 1 × M | 1 × H | 1 × T + BSA | 1 × H | 1 × H | 1 × M | 1 × H |
| EcoR V | 0.5 × K | 1 × K | 1 × H | 1 × H | 1 × H | — | 2 × T | 1 × K | 0.5 × K | 1 × K + BSA | 1 × H | 1 × H + BSA | 1 × H | 1 × K | 0.5 × K | 1 × H | 0.5 × K + BSA | 1 × H | 1 × H | 2 × T | 1 × H |
| Hinc II | 1 × M | 0.5 × K | 2 × K | 1 × M | 1 × M | 2 × T | — | 1 × M | 1 × M | 1 × M + BSA | 1 × T | 0.5 × K + BSA | 1 × M | 0.5 × K | 1 × M | 1.5 × K | 1 × T + BSA | 1 × M | 2 × T | 1 × M | 1 × M |
| Hind III | 1 × M | 1 × K | 1 × K | 1 × M | 1 × M | 1 × K | 1 × M | — | 1 × M | 1 × K + BSA | 1 × K | 0.5 × K + BSA | 1 × M | 1 × K | 1 × M | 1.5 × K | 1 × T + BSA | 1 × M | 1 × K | 1 × M | 1 × M |
| Kpn I | 1 × M | 0.5 × K | 1 × T | 1 × M | 1 × M | 0.5 × K | 1 × M | 1 × M | — | 0.5 × K + BSA | 1 × T | 0.5 × K + BSA | 1 × M | 0.5 × K | 1 × L | 1.5 × T + BSA | 1 × T + BSA | 1 × M | 0.5 × K | 1 × M | 1 × M |
| Nco I | 1 × M + BSA | 1 × K + BSA | 1 × K + BSA | 1 × K + BSA | 1 × K + BSA | 1 × K + BSA | 1 × M + BSA | 1 × K + BSA | 0.5 × K + BSA | — | 1 × K + BSA | 0.5 × K + BSA | 1 × K + BSA | 1 × K + BSA | 0.5 × K + BSA | 1.5 × T + BSA | 1 × T + BSA | 1 × K + BSA | 1 × K + BSA | 1 × M + BSA | 1 × K + BSA |
| Nde I | 1 × T | 1 × K | 1 × H | 1 × H | 1 × H | 1 × H | 1 × T | 1 × K | 1 × T | 1 × K + BSA | — | 1 × H + BSA | 1 × H | 1 × K | 1 × T | 1 × H | 1 × T + BSA | 1 × H | 1 × H | 1 × T | 1 × H |
| Not I | 0.5 × K + BSA | 0.5 × K + BSA | 1 × H + BSA | 1 × H + BSA | 1 × H + BSA | 1 × H + BSA | 0.5 × K + BSA | 0.5 × K + BSA | 0.5 × K + BSA | 0.5 × K + BSA | 1 × H + BSA | — | 1 × H + BSA | 2 × K + BSA | 0.5 × K + BSA | 1 × H + BSA | 0.5 × T + BSA | 1 × H + BSA | 1 × H + BSA | 0.5 × K + BSA | 1 × H + BSA |
| Pst I | 1 × M | 1 × K | 1 × H | 1 × H | 1 × H | 1 × H | 1 × M | 1 × M | 1 × M | 1 × K + BSA | 1 × H | 1 × H + BSA | — | 1 × K | 1 × M | 1 × H | 0.5 × T + BSA | 1 × H | 1 × H | 1 × M | 1 × H |
| Pvu I | 0.5 × K | 1 × K | 1 × K | 1 × K | 1 × K | 1 × K | 0.5 × K | 1 × K | 0.5 × K | 1 × K + BSA | 1 × K | 2 × K + BSA | 1 × K | — | 0.5 × K | 1.5 × K + BSA | 1 × K + BSA | 1 × K | 1 × K | 0.5 × K | 1 × K |
| Sac I | 1 × M | 0.5 × K | 0.5 × K | 1 × M | 1 × M | 0.5 × K | 1 × M | 1 × M | 1 × L | 0.5 × K + BSA | 1 × T | 0.5 × K + BSA | 1 × M | 0.5 × K | — | 1.5 × T + BSA | 1 × T + BSA | 1 × M | 0.5 × K | 1 × M | 1 × M |
| Sal I | 1.5 × T | 1.5 × T | 1 × H | 1 × H | 1 × H | 1 × H | 1.5 × K | 1.5 × K | 1.5 × T + BSA | 1.5 × T + BSA | 1 × H | 1 × H + BSA | 1 × H | 1.5 × K + BSA | 1.5 × T + BSA | — | 1.5 × T + BSA | 1 × H | 1 × H | 1.5 × T | 1 × H |
| Sma I | 1 × T + BSA | 0.5 × T + BSA | 1 × T + BSA | 1 × T + BSA | 1 × T + BSA | 0.5 × K + BSA | 1 × T + BSA | 1 × T + BSA | 1 × T + BSA | 1 × T + BSA | 1 × T + BSA | 0.5 × T + BSA | 0.5 × T + BSA | 1 × K + BSA | 1 × T + BSA | 1.5 × T + BSA | — | 1 × T + BSA | 0.5 × T + BSA | 1 × T + BSA | 1 × T + BSA |
| Spe I | 1 × M | 1 × K | 1 × H | 1 × M | 1 × H | 1 × H | 1 × M | 1 × M | 1 × M | 1 × K + BSA | 1 × H | 1 × H + BSA | 1 × H | 1 × K | 1 × M | 1 × H | 1 × T + BSA | — | 1 × H | 1 × M | 1 × H |
| Sph I | 0.5 × K | 1 × K | 1 × H | 1 × H | 1 × H | 1 × H | 2 × T | 1 × K | 0.5 × K | 1 × K + BSA | 1 × H | 1 × H + BSA | 1 × H | 1 × K | 0.5 × K | 1 × H | 0.5 × T + BSA | 1 × H | — | 2 × T | 1 × H |
| Xba I | 1 × M | 0.5 × K | 2 × T | 1 × M | 1 × M | 2 × T | 1 × M | 1 × M | 1 × M | 1 × M + BSA | 1 × T | 0.5 × K + BSA | 1 × M | 0.5 × K | 1 × M | 1.5 × T | 1 × T + BSA | 1 × M | 2 × T | — | 1 × M |
| Xho I | 1 × M | 1 × K | 1 × H | 1 × H | 1 × H | 1 × H | 1 × M | 1 × M | 1 × M | 1 × K + BSA | 1 × H | 1 × H + BSA | 1 × H | 1 × K | 1 × M | 1 × H | 1 × T + BSA | 1 × H | 1 × H | 1 × M | — |

- Note 1) It is confirmed that 10 units of each enzyme completely digest 1 µg of DNA at 37°C in one hour in 50 µl reaction mixture.
 2) The concentration of Glycerol should be less than 10% to minimize star activity.
 3) DNA may not be digested completely, when recognition sequences of two enzymes are close each other, or when DNA takes high-structure conformation.

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