

## Reaction Conditions:

## Buffer V4,

10 mM Tris- $\mathrm{HCl}\left(\mathrm{pH} 8.5\right.$ at $30^{\circ} \mathrm{C}$ ), $10 \mathrm{mM} \mathrm{MgCl}_{2}$,
100 mM KCl , and $100 \mu \mathrm{~g} / \mathrm{ml}$ BSA.
Incubate at $37^{\circ} \mathrm{C}$.
Dilution: Viva Buffer A 10 mM Tris- $\mathrm{HCl}\left(\mathrm{pH} 7.4\right.$ at $25^{\circ} \mathrm{C}$ ), $50 \mathrm{mM} \mathrm{KCl}, 0.1 \mathrm{mM}$ EDTA, 1 mM DTT, $200 \mu \mathrm{~g} / \mathrm{ml}$ BSA and $50 \%$ glycerol.

Thermal Inactivation: $65^{\circ} \mathrm{C}$ for 20 minutes

## Storage Buffer:

10 mM Tris-HCl (pH 7.5), $50 \mathrm{mM} \mathrm{KCl}, 0.1 \mathrm{mM}$ EDTA,
7 mM 2-mercaptoethanol, $200 \mu \mathrm{~g} / \mathrm{ml} \mathrm{BSA}$ and 50\% glycerol.

## Unit Definition:

1 u is defined as the amount of enzyme that is required to digest $1 \mu \mathrm{~g}$ of DNA in 1 hour at $37^{\circ} \mathrm{C}$ in $50 \mu \mathrm{l}$ of assay buffer.

## Quality Control Assays:

## Ligation/ Recutting Assay:

After 20-fold overdigestion with AspS9 I, more than $90 \%$ of the DNA fragments can be ligated and recut.

## Overdigestion assay:

An unaltered banding pattern was observed after $1 \mu \mathrm{~g}$ of DNA was digested with 40u of AspS9 I for 16 hours at $37^{\circ} \mathrm{C}$.

| Activity in Reaction Buffer |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: |
| V1 | V2 | V3 | V4 | V5 |
| $75 \%$ | $50 \%$ | $75 \%$ | $100 \%$ | $50 \%$ |

Buffer UB
$\lambda$ DNA (dam- \& dcm-) 1.0\% Agarose

 \begin{tabular}{|l|l|l|l|}
\hline 0.5 X \& 1.0 X \& 1.5 X \& 2.0 X <br>
\hline

 

\hline $25 \%$ \& $100 \%$ \& $100 \%$ \& $75 \%$ \& * Buffer UB is provided for double digestion purpose. <br>
\hline
\end{tabular}

## NOTE:

* Blocked by overlapping dcm-methylation (CTCWGG): GGNCCWGG
* Total reaction volume dependent on experiment.
* The amount of enzyme to be used is very much dependent on the DNA template.
* For plasmid DNA, 5-10X more enzyme is required.


## Example of Digestion Reaction

## Enzyme

: 1 unit
Lambda (dam-8dcm-) $0.3 \mu \mathrm{~g} / \mu \mathrm{l} \quad: 3.33 \mu \mathrm{l}(1 \mu \mathrm{~g} \mathrm{DNA})$
10X Reaction Buffer $5 \mu$
Sterile Distilled Water : Up to $50 \mu \mathrm{l}$

Product Use Limitation
This product is for research purposes and in vitro use only.
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