#### Restriction Endonuclease

# CERTIFICATE OF ANALYSIS

Source: Acinetobacter calcoaceticus W2131.

# AclW I

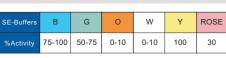
Recognition Sequence:

> E211 100 units 2.000 u/ml

GGATC(N),↓

SibEnzyme®

Lot: Exp:



BSA Dam



 $CCTAG(N)_{5}\uparrow$ 

Store at -20°C

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**Reaction Conditions:** 

10 mM Tris-HCl (pH 7.5), 100 mM KCl, 0.1 mM EDTA,

7 mM 2-mercaptoethanol, 200 µg/ml BSA, 50%

1x SE-Buffer Y, BSA (100 µg/ml). Incubate at 37° C.

1X SE-Buffer Y (pH 7.9 @ 25° C): 33 mM Tris-Ac 66 mM KAc 10 mM MqAc 1 mM DTT

### Heat Inactivation:

Supplied in:

glycerol.

Enzyme is inactivated by incubation at 65 °C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to digest 1  $\mu$ g of  $\lambda$  DNA in 1 hour at 37° C in a total reaction volume of 50 µl. To obtain 100% activity, BSA should be added to the 1x reaction mix to a final concentration of 100  $\mu$ g/ml.

## Quality Control Assays

Ligation: After 3-fold overdigestion with AclW I, ~50% of the DNA fragments can be ligated with T4 DNA Ligase and recut.

In the presence of 10% PEG ligation is better.

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 4 Units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour.

No using BSA for long incubation.

Oligonucleotide Assay: No detectable degradation of a single-stranded and double-stranded oligonucleotide was observed after incubation with 2 units of restriction endonuclease for 3 hours.

#### Enzyme Properties:

When using a buffer other than the optimal (Supplied) SE-Buffer, it may be necessary to add more enzymes to achieve complete digestion.

**Reagents Supplied with Enzyme:** 10X SE Buffer Y, BSA (10 mg/ml).

Blocked by overlapping dam methylation.