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Simplifying Genomics

TypeOne™ Restriction Inhibitor

Cat. No. TY0261H

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1. Introduction

DNA transformation can be difficult to achieve in many bacterial strains due to the presence of one or more restriction and modification (R-M) systems which cleave unmodified DNA. TypeOne™ Restriction Inhibitor significantly increases transformation efficiencies of unmodified DNA in bacterial strains with type I R-M systems.[1]* Developed as a unique preparation of a phage protein called "ocr",[2] TypeOne Inhibitor can be electroporated into cells along with transforming DNA. *In vivo*, this protein acts as a molecular decoy that blocks the DNA binding site of type I R-M enzymes and inhibits cleavage of unmodified DNA.

Type I R-M systems are widespread in Eubacteria and Archaebacteria but are not well characterized.[3] Because TypeOne Inhibitor blocks type I R-M enzymes that recognize different DNA target sequences its use does not require prior knowledge of the restriction specificity of the host or the restriction sites on the transforming DNA.

TypeOne Restriction Inhibitor is available in a 100 µg size, at a concentration of 5 µg/µl.

2. Product Specifications

Storage: Store only at –20°C in a freezer without a defrost cycle.

Storage Buffer: TypeOne Restriction Inhibitor is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 10 mM NaCl, 0.1 mM EDTA, 1.0 mM dithiothreitol, and 0.1% Triton® X-100.

Dilution Buffer: TypeOne Restriction Inhibitor may be diluted in the indicated Storage Buffer.

Quality Control: *Salmonella typhimurium* LT2 contains the StyL TIII type I R-M system that cleaves pUC19 DNA at three sites. Five micrograms (1 µl) of TypeOne Restriction Inhibitor must increase the transformation efficiency of electrocompetent *S. typhimurium* LT2 at least 100-fold (e.g., 3.0×10^6 vs. 3.0×10^8 cfu/µg of DNA) when electroporated with 100 pg of unmodified pUC19 DNA.

Contaminating Activity Assays: TypeOne Restriction Inhibitor is free of detectable RNase, endonuclease and double-stranded exonuclease activity as well as contaminating DNA.

3. Recommended Protocol

1. Add 5 µg (1 µl) of TypeOne Restriction Inhibitor and transforming DNA (amounts will vary) to 40-50 µl of electrocompetent cells. Transforming DNA may consist of circular DNAs (e.g., plasmids, cosmid, or fosmids) or an EZ-Tn5™ Transposome™.[4]
2. Electroporate, culture and plate cells using standard methods.

   Note: *The amount of TypeOne Restriction Inhibitor may be varied from 2.5-10 µg, however amounts greater than 10 µg do not improve transformation efficiencies in E. coli MG1655 or S. typhimurium LT2.*

4. References

1. Hoffman, L.M. *et al.*, (2002) *Epicentre Forum* **9** (2), 8
2. Walkinshaw, M.D. *et al.*, (2002) *Molec. Cell* **9**, 187.
3. Murray, N.E. *et al.*, (2000) *Microbiol. Molec. Biol. Rev.* **64**, 412.
4. Hoffman, L.M. *et al.*, (2000) *Genetica* **108**, 19.