

## Introduction

Recombinant Cre recombinase Tat-Cre (was purified from an E. coli strain carrying an engineered plasmid encoding Cre Recombinase from bacteriophage P1 with additional N-terminal 6XHis tag, a Tat peptide (GRKKRRQRRRPPAGTSVSL) and an NLS sequence (PKKKRKV). This cell-permeant Cre recombinase (Tat-Cre) is the most effective protein in transduction (in vivo) and subsequent recombination compared to other forms of Cre recombinases, e.g., HNC, TCH6, HC, HNCM, CH. Incubation of fibroblast reporter cells with 1  $\mu$ M Tat-Cre for 1 to 2 hours can result in transduction of 60 ~ 90% of the cells. Addition of 100  $\mu$ M choroquine to culture medium can further enhance transduction and recombination.

### **Product Information**

- **Catalog #:** EG-1001
- Accession #: YP\_006472

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• Amino Acid Sequence:

MGHHHHHHGM GAAGRKKRRQ RRRPPAGTSV SLKKKRKVSN LLTVHQNLPA LPVDATSDEV

RKNLMDMFRD RQAFSEHTWK MLLSVCRSWA AWCKLNNRKW FPAEPEDVRD YLLYLQARGL

AVKTIQQHLG QLNMLHRRSG LPRPSDSNAV SLVMRRIRKE NVDAGERAKQ ALAFERTDFD

QVRSLMENSD RCQDIRNLAF LGIAYNTLLR IAEIARIRVK DISRTDGGRM LIHIGRTKTL

VSTAGVEKAL SLGVTKLVER WISVSGVADD PNNYLFCRVR KNGVAAPSAT SQLSTRALEG

IFEATHRLIY GAKDDSGQRY LAWSGHSARV GAARDMARAG VSIPEIMQAG GWTNVNIVMN

YIRNLDSETG AMVRLLEDGD
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- Molecular Weight: 43 kDa
- **Endotoxin Level:** < 1 EU/ug
- Expression Host: E. coli
- **Purification Methods:** FPLC
- **Shipping Temperature:** ambient temperature
- Storage Buffer: 20 mM HEPES, 600 mM NaCl, 15% trehalose, 1 mM DTT, pH 7.4 @ 25°C
- **Storage Temperature**: 4 °C or -20 °C
- **Purity:** >98% by SDS-PAGE and HPLC analysis:

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# Cre Recombinase

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### **Applications:**

- \* In vitro LoxP recombination for subcloning or vector/clone engineering
- \* Transduction into cultured cells including stem cells ex Vivo

### Transduction of Cre recombinase (Tat-Cre) into cultured cells

- 1. Dissolve dried enzyme in minimal amount of culture medium or water, leave on ice for 30 min.
- 2. For some sensitive cell lines: remove inhibitory reagents (glycerol, salt etc) using a desalting column (*e.g.*, PD-10 desalting column from GE). Make sure the desalting column is pre-equilibrated with culture medium and desalting steps must be performed inside a biosafety hood to avoid contamination.
- 3. Add appropriate amount (1 to 5  $\mu$ M) of Tat-Cre to the medium and incubate up to 24 hours. Note: serum-free medium can significantly increase transduction efficiency.
- 4. Change back to normal growth medium.
- **5.** Determine transduction efficiency.

**Note**: avoid repeated freezing after hydration, store the Cre recombinase enzyme at 4 oC. For long term storage, add glycerol to 50% and store at -20 oC or -80 oC.

Heat Inactivation: 40 units at 70°C for 10 minutes