Excellgen
Protein Engineering

Cre Recombinase

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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 μM Tat-Cre for 1 to 2 hours can result in tranduction of 60 ~ 90% of the cells. Addition of 100 μM chloroquine to culture medium can further enhance transduction and recombination.

Product Information

- **Catalog #:** EG-1001 (RP-7)
- **Accession #:** YP_006472
- **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

- **Molecular Weight:** 43 kDa
- **Endotoxin Level:** < 1 EU/ug
- **Expression Host:** *E. coli*
- **Purification Methods:** FPLC
- **Size:** 1 mg
- **Shipping Temperature:** Dry ice
- **Storage Buffer**: 20 mM HEPES, 600 mM NaCl, 50% Glycerol, 200 mM Arginine, 1 mM DTT, pH 7.4 @ 25°C

  For Cre in lyophilized form, the original Storage Buffer is: 20 mM HEPES, 600 mM NaCl, 15% trehalose, 1 mM DTT, pH 7.4 @ 25°C. Before use, dissolve the dried powder in minimal amount of serum-free culture medium or sterilized endotoxin-free water **aseptically**, leave on ice for 30 min, mix gently by pipetting.

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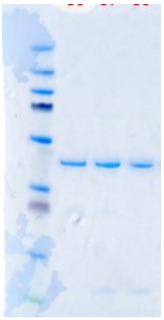
- **Storage Temperature**: –80 °C or -20 °C

- **Purity:** >98% by SDS-PAGE and HPLC analysis:



Applications:

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

Reaction Conditions: 1X Cre Recombinase Reaction Buffer, Incubate at 37°C.

In vitro Recombination Protocol

1. Prepare the Creator reaction mixture as follows:

400 ng Donor Vector (containing insert of interest)
400 ng Acceptor Vector
1 µL 10 X Cre Reaction Buffer
1 µL 10 X BSA (1 mg/ml)
2 µL Cre recombinase
dH20 up to 10 µl

A master mix of reaction buffer, BSA, ddH20, and Cre (and donor or acceptor if applicable) should be used.

2. Mix well by gently tapping the tube. Centrifuge briefly.

3. Incubate at room temperature for 15 minutes. Do not extent incubation past 15 minutes. Competing recombination reactions can reduce the yield of desired recombinants.

4. Incubate the tube at 70 °C for 5-10 minutes to stop the reaction.

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5. Transform competent cells (> 1 X 10^8 cfu/mg) with 1 ml of the reaction. Plate the transformation on an LB-agar plate containing 30 mg/ml chloramphenicol and 7% sucrose (w/v) to select for the correct recombinant vector. Allow the plates to dry for 10 minutes before inverting them.

6. Transforming into cells at 10^7-10^8 cfu/mg should yield about 5 to 10 colonies.

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

1. For some sensitive cell lines: remove inhibitory reagents (glycerol, salt etc) using a desalting column (*e.g.,* PD-10 d*esalting 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.*
2. Add appropriate amount (1 to 5 μM) of Tat-Cre to the medium and incubate up to 24 hours. Note: serum-free medium can significantly increase transduction efficiency.
3. Change back to normal growth medium.
4. Determine transduction efficiency.

1X Cre Recombinase Reaction Buffer: 50 mM Tris-HCl, 33 mM NaCl, 10 mM $MgCl_2$ pH 7.5 @ 25°C.

10X Cre Recombinase Reaction Buffer: 500 mM Tris-HCl, 330 mM NaCl, 100 mM $MgCl_2$ pH 7.5 @ 25°C.

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