

## Introduction

Recombinant Cre recombinase NLS-Cre was purified from an *E. coli* strain carrying an engineered plasmid encoding Cre Recombinase from bacteriophage P1 with additional N-terminal 6XHis tag and an NLS sequence (PKKKRKV). Incubation of fibroblast reporter cells with 1~5  $\mu$ M HTNC for 1 to 2 hours can result in transduction of ~60 % of the cells. Addition of 100  $\mu$ M choroquine to culture medium may enhance transduction and recombination.

## Product Information

- **Catalog #:** EG-1067
- **Accession #:** YP\_006472
- **Amino Acid Sequence:**  
MGSSHHHHHPKKRKVSNLLTVHQNLPALPVDATSDEVKRLMDMFRDRQAFSEHTWKM  
LLSVCRSWAAWCKLNNRKWFPAEPEDVRDYLLYLQARGLAVKTIQQHLGQLNMLHRRSGL  
PRPSDSNAVSLVMRRIRKENVDAGERAKQALAFERTDFDQVRSLMENS DRCQDIRNLAFL  
GIAYNTLLRIAIEIARIRVKDISRTDGGRLIHIGRTKTLVSTAGVEKALSLGVTKLVERW  
ISVSGVADDPNNYLCFRVRKNGVAAPSATSQLSTRALEGIFEATHRLIYGAKDDSGQRYL  
AWSGHSARVGAARDMARAGVSIPEIMQAGGWTNVNIVMNYIRNLDSETGAMVRLLEDGD
- **Molecular Weight:** 43 kDa
- **Endotoxin Level:** < 1 EU/ $\mu$ g
- **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:** -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*

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

1. Dissolve dried enzyme in minimal amount of culture medium or water at 4 oC.
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.*
1. Add appropriate amount (1 to 5  $\mu$ M) of HNC-Cre to the medium and incubate up to 24 hours. Note: serum-free medium can significantly increase transduction efficiency.
2. Change back to normal growth medium.
3. 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