



LD Biopharma, Inc.
9924 Mesa Rim Road Suite B
San Diego, CA 92121
Tel: 858-876-8266
<http://www.ldbiopharma.com>

- PRODUCT DATA SHEET -

Name of Product: Recombinant Cre-11R Protein
Catalog Number: vRP-0124
Manufacturer: LD Biopharma, Inc.

Introduction

Cre-Lox recombination is a specific type of site-specific recombination developed by Dr Brian Sauer. Cre-Lox recombination involves the targeting of a specific sequence of DNA and splicing it with the help of an enzyme called Cre recombinase. Cre recombinase, often abbreviated to Cre, is a type I topoisomerase from P1 bacteriophage that catalyzes site specific recombination of DNA between LoxP site. Cre does not require any energy cofactors and Cre-mediated recombination quickly reaches equilibrium between substrate and reaction products. To efficiently delivery Cre protein into target cells without using Cre DNA or expression plasmid, we have modified Cre protein with SV40 large T nuclei translocation signal (NTS) domain, then fusion with 11 arginine (11R) domain to create cell membrane penetrable recombinant Cre-11R protein, which could be directly incubated in medium to induce Cre-Lox recombination for target cells. To achieve a true function for intracellular delivery recombinant Cre-11R protein, this recombinant protein has to enter cell membrane, escape from endosome trapping, migrate into nuclei to form tetramer for perform site-specific recombination. Combined with 293-LoxP-eGFP cell line, recombinant Cre-11R protein provides an excellent protein delivery testing for monitoring intracellular delivery efficiency.

Full-length Cre gene was constructed with 29aa N-terminal T7 tag/His/TEV cleavage site tag and C-terminal 11R tag. This protein was expressed in *E. coli* and chromatographically purified. To achieve the best intracellular protein delivery efficiency, ProFectin™ (protein delivery reagent) reagent should be used in serum free medium for 30 min incubation.

Gene Symbol: Cre
Accession Number: YP_006472
Species: Bacteriophage P1
Size: 50 µg / Vial
Composition: 0.5 mg/ml, sterile-filtered, in 20 mM pH 8.0 Tris-HCl Buffer, with 100 mM NaCl, 10 mM KCl, 1 mM DTT and 10% Glycerol.
Storage: In Liquid. Keep at -80°C for long term storage. Product is stable at 4 °C for at least 7 days.



LD Biopharma, Inc.
9924 Mesa Rim Road Suite B
San Diego, CA 92121
Tel: 858-876-8266
<http://www.ldbiopharma.com>

Key References

James J. Cronica, et al. *Protein delivery of functional protein into mammalian cells in vitro and in vivo using a supercharged protein*. ACS Chem. Biol DOI: 10.1021/ cb1001153. June 14 (2010)

Hongyan Zhou, et al. *Generation of induced pluripotent stem cells using recombinant protein*. Cell Stem cell. Vol 4. Issue 5: 381-384 (2009)

Applications

1. Protein intracellular transduction for in vitro Cre-loxP recombination.
2. Negative control for 11R-Tag human transcription factor mediated cell differentiation assay in vitro.

Quality Control

1. Purity: > 90% by SDS-PAGE.
2. Functional Assay: Mix 1 μ g Cre-11R protein with 2 μ l ProFectinTM (LD Biopharma, Inc. Catalog # R-2000) by following ProFectinTM manufacture suggested protocol, and incubate transfection mixture on 0.25 x10⁶ 293-LoxP-eGFP cell line / well (6 well plate) for 30 min in serum free medium, will achieve > 35% transduction efficiency. (Positive for GFP cells).

Note: More info about ProFectinTM reagents kit, could be found at <http://www.ldbiopharma.com/html/product316.html>.

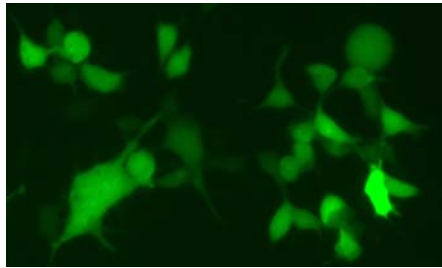
Suggested Protocol

1. Seed 0.25 x10⁶ 293-LoxP-eGFP report cell line / well (6 well plate) in 10% FCS + DMEM medium, incubate for 1 day before protein transfection.
2. Pre-warm serum free DMEM medium to 37°C, and add culture cell to 1ml serum free DMEM medium before transfection.
3. Following ProFectinTM suggested protocol to prepare transfection mixture: a) dilute 1 μ g Cre-11R protein in 60 μ l PBS buffer and mix well, b) add 2 μ l ProFectin reagent directly into Cre-11R PBS solution, c) then mix well and incubate at room temperature for 15 min.



LD Biopharma, Inc.
9924 Mesa Rim Road Suite B
San Diego, CA 92121
Tel: 858-876-8266
<http://www.ldbiopharma.com>

4. Load transfection mixture to cell sample, and incubate cells at 37°C for 30 min in serum free medium.
5. Adding 2 ml pre-warm 10% FCS+DMEM medium and cultivate cell overnight before analyzing GFP positive cells.



0.25 x 10⁶ 293-LoxP-eGFP report cell line was treated with 1ug Cre-11R protein using ProFectin reagent by following manufacture's standard protocol for 30 min and continue cultivating cells overnight before viewing GFP positive cells.

Recombinant Protein Sequence

MASMTGGQQMGRGHHHHHGNLYFQGGFPPK~~KKR~~KVSNLLTVHQNLPALPVDATSDEVK~~NLM~~
DMFRDRQAFSEHTWKMLLSVCRSWAAWCKLNNR~~KWF~~PAEPEDVRDYLLYLQARGLAVKTIQQHL
GQLNMLHRRSGLPRPSDSNAVSLVMRRIRKENVDAGERAKQALAFERTDFDQVRSLMENS~~DRCQ~~
DIRNLAFLGLIAYNTLLRIAETARIRVKDISRTD~~GGR~~MLIHIGRTKTLVSTAGVEKALSLGVTKL
VERWISVSGVADDPNNYLF~~CR~~VRKNGVAAPSATSQ~~L~~STRALEGIFEATHRLIYGAKDDSGQRYL
AWSGHSARVGAARDMARAGVSIPEIMQAGGWTN~~VNI~~VMNYIRNLDSETGAMVRLLEDGDLEESG
GGSPGRRRRRRRRRRR