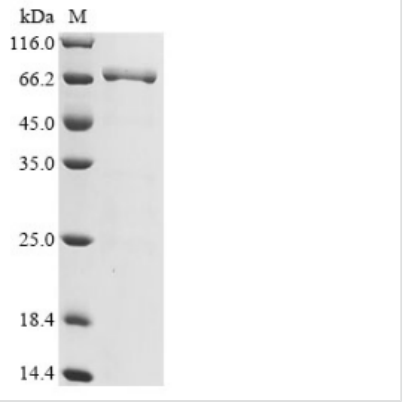
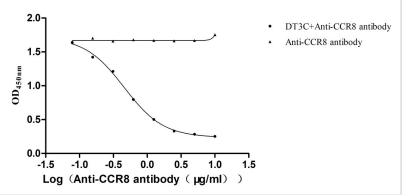
Recombinant DT3C (Diphtheria toxin & spg 3C domain) for Antibody Internalization Assay (Active)

Product Code	CSB-EP360556CQR1
Abbreviation	Recombinant Corynephage beta DT3C protein (Active)
Storage	The shelf life is related to many factors, storage state, buffer ingredients, storage temperature and the stability of the protein itself. Generally, the shelf life of liquid form is 6 months at -20°C/-80°C. The shelf life of lyophilized form is 12 months at -20°C/-80°C.
Uniprot No.	P00588/P19909
Form	Lyophilized powder
Storage Buffer	Lyophilized from a 0.2 μm filtered PBS, 6% Trehalose, pH 7.4
Product Type	Recombinant Protein
Immunogen Species	N/A
Biological Activity	①After DT3C 10μg/ml formed complexes with different concentrations of Anti-CCR8 antibodies (CSB-RA004847MA3HU), cytotoxicity experiments were conducted on CHOK1/Human CCR8 cells (CSB-SC004847HU2). The ED50 is 0.3708-0.5608 μg/ml.②After DT3C 10μg/ml formed complexes with different concentrations of Anti-CCR8 antibodies (CSB-RA004847MA3HU), cytotoxicity experiments were conducted on CT26/Human CCR8 cells (CSB-SC004847HU3). The ED50 is 0.4661-0.8614 μg/ml.
Purity	Greater than 90% as determined by SDS-PAGE.Greater than 90% as determined by SEC-HPLC.
Source	E.coli
Target Names	DT3C
Notes	Repeated freezing and thawing is not recommended. Store working aliquots at 4°C for up to one week.
Tag Info	N-terminal 6xHis-tagged
Mol. Weight	69.4 kDa
Protein Length	Linked Heterodimer
Image	



(Tris-Glycine gel) Discontinuous SDS-PAGE (reduced) with 5% enrichment gel and 15% separation gel.



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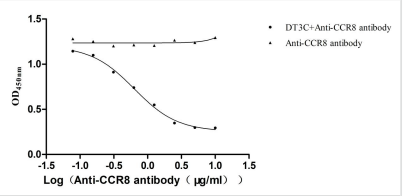

Activity
After DT3C 10μg/ml formed complexes with different concentrations of Anti-CCR8 antibodies (CSB-RA004847MA3HU), cytotoxicity experiments were conducted on CT26/Human CCR8 cells (CSB-SC004847HU3). The ED_{50} is 0.4661-0.8614 μg/ml.

Description

The recombinant DT3C protein was produced by expressing a DNA fragment encoding the catalytic and translocation domains of the Corynephage beta diphtheria toxin (DT) (33-417aa) fused with the Streptococcal protein G-derived 3C domain (291-497aa) in E.coli. The 6xHis-tag sequence was placed in the N-terminus of the DT3C encoding sequence. The resulting recombinant DT3C protein consists of diphtheria toxin (DT) lacking the receptor-binding domain but containing the C1, C2, and C3 domains of Streptococcus protein G (3C) [1]. The purity of the recombinant DT3C protein was assessed using SDS-PAGE, demonstrating a purity level of up to 90%. On the gel, DT3C migrated as a band with an approximate molecular weight of 69.4 kDa.

The rationale behind designing the recombinant DT3C protein stems from the cytotoxic property of diphtheria toxin, which inhibits the protein translation machinery in cells, and the Fc-binding domain (3C), capable of binding to any IgG antibodies across different species [1][2]. It binds to a mAb to form a mAb-DT3C conjugate after incubation at room temperature, which functions in vitro similarly to an antibody-drug conjugate (ADC), delivering the cytotoxic effects of diphtheria toxin specifically to targeted cells. Moreover, the mAb-DT3C conjugate serves as a tool to assess the efficiency of mAb internalization by cells by monitoring the extent to which it induces cell death. Additionally, the recombinant DT3C protein facilitates the evaluation of cancer cells' internalization efficiency of the mAb in vitro, aiding in the screening process for

more effective monoclonal antibodies.

References:
[1] Hamamichi, S., Fukuhara, T., et al. Novel method for screening functional antibody with comprehensive analysis of its immunoliposome [J]. Sci Rep 11, 4625 (2021).
[2] Miki Yamaguchi, Yukari Nishii, et al. Development of a sensitive screening method for selecting monoclonal antibodies to be internalized by cells [J]. Biochemical and Biophysical Research Communications, Volume 454, Issue 4, 28 November 2014, Pages 600-603.

Endotoxin	Less than 1.0 EU/ug as determined by LAL method.
Reconstitution	We recommend that this vial be briefly centrifuged prior to opening to bring the contents to the bottom. Please reconstitute protein in deionized sterile water to a concentration of 0.1-1.0 mg/mL.We recommend to add 5-50% of glycerol (final concentration) and aliquot for long-term storage at -20°C/-80°C. Our default final concentration of glycerol is 50%. Customers could use it as reference.
Shelf Life	The shelf life is related to many factors, storage state, buffer ingredients, storage temperature and the stability of the protein itself. Generally, the shelf life of liquid form is 6 months at -20°C/-80°C. The shelf life of lyophilized form is 12 months at -20°C/-80°C.