Recombinant DT3C Protein

Please read the manual carefully before use

Cat. No. FC501

Version: Version 1.0

Storage: at -70°C or below for one year, at -18°C or below for one month, and at 2°C-8°C for two weeks. Avoid repeated freeze-thaw cycles. It is recommended to aliquot the product into smaller portions and store at -70°C or below.

Description

DT3C is a recombinant fusion protein composed of the diphtheria toxin (DT) lacking its receptor-binding domain, fused to the C1, C2, and C3 IgG-binding domains of Streptococcal protein G. This protein binds with high affinity to the Fc region of antibodies. Upon internalization into cells along with the antibody, DT3C is cleaved by intracellular furin protease, releasing the toxic DT moiety. DT inhibits EF2 through ADP-ribosylation, thereby blocking intracellular protein synthesis and ultimately inducing cell death. Free DT3C that does not enter cells lacks cytotoxic activity. Therefore, the extent of cell killing directly reflects the efficiency of antibody internalization.

This product is expressed in *Escherichia coli*, comprises amino acids 33-417 (P00588) and 291-497 (P19909), contains an N-terminal 6×His tag, has a molecular weight of 69 kDa, a purity of ≥90% (SDS-PAGE), a concentration of 1 mg/ml, and an endotoxin level of <1 EU/μg.

The use of recombinant DT3C protein is straightforward: simply incubate DT3C with the target antibody for 30 minutes to form the Ab-DT3C complex. This complex requires no further processing and can be directly added to cells in complete culture medium for incubation. *In vitro*, it functions analogously to antibody-drug conjugates (ADCs), delivering the cytotoxic effects of diphtheria toxin to specific cells through internalization, thereby enabling the assessment of antibody internalization efficiency.

Kit Contents

Component	FC501-01	FC501-02
Recombinant DT3C Protein	100 μg	1 mg

Procedure

(1) Harvest target antigen-expressing cells in the logarithmic growth phase. Digest, count, and resuspend the cells in culture medium containing 20% serum to the desired cell density.

(2) Seed the cells into a 96-well plate at 100 μl per well. Incubate overnight in a cell culture incubator to allow the cells to adhere.

2. Antibody-DT3C Conjugate Preparation

Dilute the DT3C protein and the antibody using serum-free basal medium, and mix at a certain ratio and concentration. Incubate the mixture in a 37°C incubator for 30 minutes to form conjugates. Perform serial dilutions of the conjugates.

3. Co-incubation of Cells with Conjugates

(1) Remove 50 μl of the cell culture supernatant.

(2) Add 50 μl per well of the serially diluted conjugate mixtures to the 96-well plate sequentially.

(3) Return the plate to the incubator and continue culturing for 48-72 hours.

4. Detection

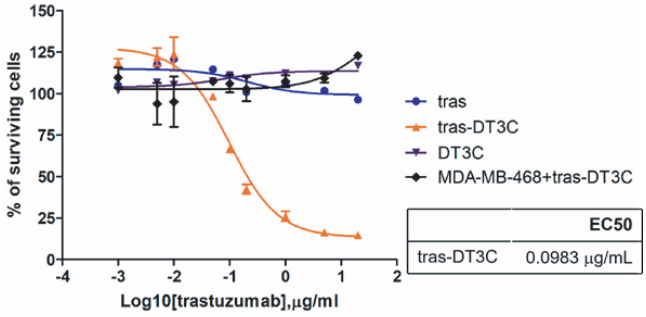
Measure cell viability using a luminescent cell viability assay kit. Add 100 μl of detection reagent to each well, incubate for 10 minutes, and then read the luminescence values using a microplate reader.

Result

[Trastuzumab Internalization Assay]

Trastuzumab specifically recognizes the Her2 antigen. In this experiment, SK-BR-3 human breast cancer cells (high Her2 expression) were used for antibody internalization efficiency testing, while MDA-MB-468 human breast cancer cells (Her2-negative) served as a negative control. The molar ratio of trastuzumab to DT3C was 1:2, with 7,000 cells per well, and

detection was performed after 72 hours of co-incubation. The results are as follows: In Her2-negative cells, the antibody-DT3C conjugate showed no significant killing effect. In Her2-positive cells, treatment with trastuzumab or DT3C alone had no significant effect. Cell death was only observed when the antibody-DT3C conjugate was added, indicating that the conjugate was internalized via antibody-mediated endocytosis, releasing the toxic DT3C component. The cell killing rate reflects the internalization efficiency of the antibody.



Notes

1. Conjugation Incubation Conditions

Serum contains high levels of immunoglobulin G (IgG), which can interfere with the binding between DT3C and the target antibody. Therefore, the conjugation incubation step must be performed in serum-free basal medium. After conjugates are formed, they can be added to complete culture medium containing serum and antibiotics for cell culture.

2. Optimal Concentration of DT3C and Optimal Ratio with Antibody

Theoretically, one antibody molecule can bind two DT3C molecules, i.e., the molar ratio of antibody to DT3C is 1:2. However, actual internalization efficiency is influenced by various factors such as cell type, antigen expression level, antibody epitope, and affinity. Therefore, the optimal ratio should be determined in preliminary experiments. The recommended approach is as follows: maintain a fixed DT3C concentration while titrating the antibody to determine the optimal concentration for internalization; subsequently, evaluate different antibody-to-DT3C molar ratios to identify the most effective DT3C concentration.

Product Recommendation

TransDetect® Luminescent Cell Viability Detection Kit is a ready-to-use, single-component reagent. Add an equal volume of the reagent directly to the cell culture, mix, and measure after 10 minutes. The procedure is simple and rapid. The kit offers high sensitivity and a wide linear range, with good linearity from 10 to 100,000 cells. The "glow-type" luminescent signal is stable with a half-life of over 3 hours, suitable for both small-scale assays and high-throughput screening applications.

Cat. No.	Product Name	Signal Half-Life
FC401	*TransDetect*® Luminescent Cell Viability Detection Kit	>3 hours

For research use only, not for clinical diagnosis.

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