bsAb CD3-CD28 for T cell expansion

Bispecific antibody (bsAb) against human CD3 and human CD28

Catalog codes: bsab-tex-1, bsab-tex-2, bsab-tex-4

For research use only

Version 24J01-NJ

PRODUCT INFORMATION

Contents: bsAb CD3-CD28 is a purified bispecific antibody (bsAb), provided azide-free and lyophilized. It is available in three quantities:

- **bsab-tex-1:** 100 µg
- **bsab-tex-2:** 2 x 100 µg
- **bsab-tex-4:** 4 x 100 µg

Target: Human (h)CD3 (clone OKT3) and hCD28 (clone 15E8)
Format: IgG-like $(scFv)_2$-$(scFv)_2$-Fc molecule
Source: Chinese hamster ovary (CHO) cells
Capacity: 100 µg of bsAb CD3-CD28 is sufficient to activate and expand up to $1x10^8$ enriched T cells or peripheral blood mononuclear cells (PBMCs), when used at the recommended titer of 1:100.

Storage and stability

- Product is shipped at room temperature. Upon receipt, store lyophilized antibody at -20 °C. Lyophilized product is stable for at least 1 year.
- Reconstituted antibody is stable for 1 year when aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

Quality control

- Biological activity has been confirmed using cellular assays.
- The absence of bacterial contamination (e.g. endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

Antibody resuspension (0.1 mg/ml)

Note: Ensure you see the lyophilized pellet before resuspension.

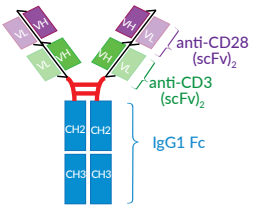
- Add 1 ml of sterile H_2O to the vial and gently pipette until completely resuspended.

APPLICATION

The bsAb CD3-CD28 is a bispecific antibody (bsAb) designed to activate and expand enriched T cell populations or resting T cells from PBMCs. T cell expansion is achieved by culturing for up to 1-2 months.

PRODUCT INFORMATION

The bispecific antibody (bsAb) CD3-CD28 is a fusion protein dimer comprising tandem single-chain variable fragments $(scFv)_2$ from two monoclonal antibodies (mAbs). These mAbs were derived from the clones OKT3 and 15E8 targeting the human CD3 and CD28 receptors, respectively. This so-called $(scFv)_2$-$(scFv)_2$-Fc molecule belongs to the family of IgG-like antibodies. It features an IgG1 Fc fragment, which contributes to increased solubility, serum half-life, as well as facilitated purification.



CELLULAR ASSAY

All steps in the protocol have to be performed under sterile conditions.

Note: For optimal T cell expansion, a daily inspection of culture is required.

Required Cell Culture Medium

- **Growth Medium:** RPMI, 10% (v/v) heat-inactivated FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, 50 µM β-mercaptoethanol, 1 mM Sodium pyruvate, 1x NEAA

Required Conditions

- 5% CO_2, 37°C Incubator

Required Supplements

- Recombinant human IL-2 cytokine

Day 1: T cell isolation and activation

1. Isolate T cells according to your protocol.
2. Determine cell number.
3. Resuspend T cells at $1x10^6$ cells/ml in freshly prewarmed media *(see table on page 2)*.
4. Add recombinant human IL-2 (final concentration 20 IU/ml) and bsAb CD3-CD28 (final concentration **1 µg/ml**) directly to the cells.
5. Incubate at 37 °C, 5% CO_2 for **2 days.**

Day 3: Stop activation

1. Remove residual reagent bsAb CD3-CD28 by centrifugation at 300 x g (RCF) for 5 min.
2. Aspirate supernatant.
3. Determine cell number.
4. Resuspend T cells at $1x10^6$ cells/ml in freshly prewarmed medium.
5. Add recombinant human IL-2 (final concentration 20 IU/ml).
6. Incubate at 37 °C, 5% CO_2 for **2-3 days.**

Day 5/6: Expansion

1. Centrifuge cells at 300 x g (RCF) for 5 min.
2. Aspirate supernatant.
3. Determine cell number.
4. Resuspend T cells at $1x10^6$ cells/ml in freshly prewarmed medium.
5. Add recombinant human IL-2 (final concentration 20 IU/ml).
6. Incubate at 37 °C, 5% CO_2 for **2-3 days.**
7. Return to Day 1 for another cycle of activation/expansion.

This activation cycle can easily by performed up to 1 month with the same cell population, if the activation is repeated every 7-8 days.

RESTRICTION USE

This antibody is distributed for research purposes only. It is not intended for diagnosis or therapeutic use.

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CULTURE CONDITIONS FOR T CELLS

Culture plate	Volume medium	Total cell number	bsAb CD3-CD28 (stock 0.1 mg/ml) to add per well
48 well	1 ml	$1X10^6$	10 µl
24 well	2 ml	$2X10^6$	20 µl
12 well	3 ml	$3X10^6$	30 µl
6 well	5 ml	$5X10^6$	50 µl
T25	10 ml	$1X10^7$	100 µl
T75	20 ml	$2X10^7$	200 µl

Table 1: *Optimal surface density when working with purified T cells*

RELATED PRODUCTS

Product	Description	Cat. Code
Recombinant human IL-2	Recombinant cytokine	rcyc-hil2
Normocin®	Antimicrobial reagent	ant-nr-1
Primocin®	Antimicrobial reagent	ant-pm-05

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