Human CD3/CD28 T Cell Activation Beads

Introduction

Human CD3/CD28 T Cell Activation Beads are inert superparamagnetic beads covalently conjugated to Anti-CD3 and Anti-CD28 antibodies. These beads are commonly used to activate and expand T cells without feeder cells (antigen-presenting cells) or antigens. T cell expansion can be stimulated by the addition of recombinant human IL-2 during cell culture. After T cell activation or expansion, the beads can be removed by magnets.

This kit is ideal for T cell activation in peripheral blood mononuclear cells (PBMCs) or T cell subsets (e.g., $CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells).

Components and Storage

Components \ Size	1 mL (for 10^8 cells)	Storage
Human CD3/CD28 T Cell Activation Beads	1 mL	4°C
Shipping: Blue ice	Shelf life: 1 year	

Properties

antibody	Anti-CD3 antibody and Anti-CD28 antibody
Magnetic bead concentration	1×10^8 Beads/mL
Bead particle size	5 μm
stockpile	PBS with 0.1% BSA and 0.1% proclin-300, pH 7.4

Protocol

Take sorting human $CD3^+$ T cells for example

1. Preparation before the experiment

   1) Wash buffer: PBS containing 2 mM EDTA and 2% fetal bovine serum (FBS), where 2% FBS can be replaced with 0.5% BSA. Wash buffers need to be sterilized by filtering through a 0.22 μm membrane.

   2) Culture medium: RPMI1640 containing 2 mM glutamine and 10% FBS, with or without penicillin/streptomycin at your option. When T cells need to be expanded, add recombinant human IL-2 to the medium with a final concentration of 30 U/mL. Commercial T cell culture media can also be used directly.

2. Wash the beads

1) Resuspend the Human CD3/CD28 T Cell Activation Beads in the vial thoroughly by vortexing > for 30 sec or rotating for 5 min.

2) Transfer the desired volume of beads to a tube, and add an equal volume of wash buffer. If the volume of beads is less than 1 mL, add 1 mL of wash buffer. Vortex for 5 sec or mix well with a pipette, taking care not to create bubbles. (It can also be washed directly with culture medium).

3) Place the tube on a magnet for 3 min and discard the supernatant.

4) Repeat steps 2-3, this time using culture medium to wash the beads.

5) Resuspend the beads using 40-fold the initial volume. If washing 25 μL of beads, resuspend beads with 1 mL of medium. The final concentration of beads is 2.5×10^6 beads/mL.

3. Human T cell activation (96-well plate as an example)

1) Adjust the T cell concentration to 1×10^7 cells/mL with the medium. Add 25 μL of T cells per well, resulting in 2.5×10^5 cells per well. Add 75 μL of medium to each well, resulting in a final volume of 100 μL per well. The number of cells seeded can be adjusted according to the specific experiment to maintain a final volume of 100 μL.

2) Add 100 μL of washed beads to each well to obtain a 1:1 ratio of beads to cells. The ratio of beads to cells can be adjusted according to the specific experiment.

3) Culture cells and beads in a 37°C, 5% CO_2 incubator according to your specific experiment.

4) Harvest activated T cells for downstream experiments.

4. Human T cell expansion

1) Adjust the $CD3^+$ T cell with culture medium to a density of $1\text{-}1.5\times10^6$ cells/mL. Add the washed beads in a 1:1 ratio of beads to cells.

2) After 2 days of activation, add recombinant human IL-2 to the medium with a final concentration of 30 U/mL.

3) Culture cells and beads in a 37°C, 5% CO_2 incubator according to your specific experiment.

4) Examine cells daily, noting cell size and shape. When cells shrink or proliferate significantly more slowly, it is a sign that the cells may be depleted.

5) It is recommended not to treat the cells for the first two days of cell activation and to observe the cells regularly after 2 days. When the medium turns yellow, replace medium with a fresh culture medium with IL-2.

6) Count cells regularly, and when the cell density exceeds 2.5×10^6 cells/mL, gently pipette and mix well,

and adjust the cell concentration to 0.5-1×10^6 cells/mL

Note

1. Do not freeze this product.
2. This product should be avoided from drying during use and storage, and should not be placed in a magnet for a long time to avoid agglomeration of beads and reduce the activity of beads.
3. Try to use low-adsorption pipette tips and tubes to avoid loss due to adsorption.
4. This product can be removed by a magnet, for example QuickSort Magnet (Cat. No. CS1010).
5. For your safety and health, please wear lab coats and gloves during the experiment.
6. For research use only. Not to be used in clinical diagnostic or clinical trials.

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