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Human CD3/CD28 T Cell Activation Magnetic Beads

1 Packing list

Components	HY-K0353-1 mL (For 10^8 cells)
Human CD3/CD28 T Cell Activation Magnetic Beads	1 mL

2 Introduction

The activation of T cells typically requires TCR/CD3 signals and CD28 co-stimulation signals. TCR/CD3 signaling initiates the initial activation signal through the interaction of the T cell receptor (TCR) on the T cell surface with antigen-presenting molecules on antigen-presenting cells (APCs). The CD28 co-stimulation signal is provided by the binding of CD28 on the T cell surface to B7 molecules (such as CD80, CD86) on the APCs, which enhances T cell activation, proliferation, and survival.

MCE Human CD3/CD28 T Cell Activation Magnetic Beads are based on the two important co-stimulatory signals, CD3 and CD28. The anti-CD3 and anti-CD28 antibodies conjugated to the surface of the magnetic beads can respectively activate TCR and CD28 co-stimulatory signals, without relying on feeder cells (antigen-presenting cells) or antigens, enabling simple and rapid T cell activation. During cell culture, recombinant human IL-2 can be added to stimulate T cell proliferation. After activation or expansion, the magnetic beads can be removed using a magnetic separator.

This product is suitable for the activation of peripheral blood mononuclear cells (PBMCs) or T cell subsets (such as $CD3^+$ T cells, $CD4^+$ T cells, and $CD8^+$ T cells).

3 Characteristics

Composition	Magnetic Beads
Ligand	Anti-CD3 antibody, Anti-CD28 antibody
Bead Concentration	1×10^8 Beads/mL
Bead Diameter	5 μm
Storage Solution	PBS, 0.1% BSA, 0.1% Proclin-300, pH 7.4

4 General Protocol

Recommended Buffer	
Wash Buffer	PBS, 0.5% BSA, 2 mM EDTA, pH 7.0 - 7.4
Cell Culture Medium	RPMI 1640, 10% FBS

Note: a. BSA can be replaced with 2% fetal bovine serum (FBS).

b. The wash buffer is recommended to be prepared with ultrapure water, filtered through a 0.22 μm membrane for sterilization, and stored at 4°C.

c. Recommended MCE RPMI 1640 product: HY-K3004. If the medium does not contain glutamine, it should be supplemented with glutamine to a final concentration of 2 mM.

d. The cell culture medium may optionally be supplemented with penicillin-streptomycin.

Magnetic Beads Pre-treatment (Example for a 96-well Plate)

1. Beads Preparation: Fully resuspend the magnetic beads (e.g., vortex for > 30 s or place on a rotator for > 5 min).
2. Washing the Magnetic Beads: Transfer 25 μL of magnetic beads into a sterile flow tube and add 1 mL of wash buffer, vortex for 5 s or mix thoroughly by pipetting. Place the tube on a magnetic separator for 3 min and discard the supernatant. Repeat the washing step once with cell culture medium. Resuspend the magnetic beads in 1 mL of cell culture medium, with the bead concentration is 2.5×10^6 beads/mL,which is sufficient for 10 wells in a 96-well plate.

   Note: a. Be sure to avoid the formation of bubbles during the washing process.
   b. If the initial beads volume is >1 mL, ensure the volume of wash buffer matches the beads volume during washing.
   c. It is recommended to adjust the beads volume according to the number of wells and the cell count.

Human T Cell Activation (Example for a 96-well Plate)

1. Adjust the concentration of T cells to 1×10^7 cells/mL using culture medium. Add 25 μL of T cells and 75 μL of cell culture medium to each well, resulting in 2.5×10^5 cells/well. The final volume is 100 μL/well.

   Note: The cell inoculation amount can be adjusted according to the experiment, ensuring the final volume remains 100 μL/well.
2. Add 100 μL of washed magnetic beads to each well, with 2.5×10^5 beads per well. The bead-to-cell ratio should be 1:1, and the final volume per well will be 200 μL.

   Note: The Bead-to-Cell ratio can be adjusted according to the experiment, but a ratio of 1:1 is recommended.
3. Incubate the plate in a 37°C, 5% CO_2 incubator for activation.

   Note: The incubation time for activation can be adjusted according to the needs of the experiment.
4. After activation, harvest the T cells for subsequent cell biology experiments.

Human T Cell Expansion (Example for a 96-well Plate)

1. Adjust the concentration of $CD3^+$ T cells to 1×10^6 - 1.5×10^6 cells/mL, and add an appropriate amount of pre-washed magnetic beads according to a bead-to-cell ratio of 1:1. Incubate the cells in the culture incubator for activation.
2. After 2 days of activation, add recombinant human IL-2 to the culture medium, with a final concentration of 30 U/mL. Incubate the plate in a 37°C, 5% CO_2 incubator to stimulate T cell expansion.

   Note: a. It is recommended to observe the cell activation and expansion daily, paying attention to cell morphology and size. If cells become shrinked or the proliferation rate slows down significantly, the cells may be exhausted.
   b. It is recommended not to perform any additional treatments on the cells within 2 days of activation. After 2 days, monitor the cell status regularly. Change the medium or passage the cells when the medium turns yellow or when the cell density in the plate is too high.
   c. After every medium change or passage, supplement recombinant human IL-2 to a final concentration of 30 U/mL.
3. Perform regular cell counting. When the cell density exceeds 2.5×10^6 cells/mL, gently mix the cells by pipetting and adjust the density to 0.5×10^6 - 1×10^6 cells/mL.

5 Storage Condition

4°C, 2 years

Do not freeze the magnetic beads

6 Precautions

1. When using commercial T cell culture medium for T cell expansion, the concentration of growth factors can be adjusted according to the experimental requirements.
2. It is recommended to use low-adsorption consumables, such as pipette tips and centrifuge tubes to minimize loss of magnetic beads and antibodies due to adsorption.
3. During use and storage, magnetic beads should not be subjected to high-speed centrifugation, drying, or freezing. Avoid leaving magnetic beads in a magnetic field for extended periods, as this can cause bead aggregation, reducing their binding activity.
4. Before drawing magnetic beads from the storage tube, thoroughly mix them by gentle vortexing. During operation, handle gently and avoid creating air bubbles.
5. This pruduct should be used with a magnetic separator.
6. Perform all experimental procedures gently to avoid mechanical damage to the cells.
7. This product is for R&D use only, not for drug, household, or other uses.
8. For your safety and health, please wear a lab coat and disposable gloves to operate.