Human CD3/CD28 T Cell Activation Beads

Catalog No.: BRC0010

Product Description

Human CD3/CD28 T Cell Activation Beads enable simple and rapid activation and expansion of T cells without the need for feeder cells (antigen-presenting cells) or antigens. The beads are 5 μm inert superparamagnetic particles, similar in size to antigen-presenting cells, and are covalently conjugated with anti-CD3 and anti-CD28 antibodies. Recombinant human IL-2 can be added during cell culture to stimulate T cell population expansion. The beads can be easily removed using a magnetic stand after activation or expansion.

Kit Components

Product Details	Size1 (For 1×10^8 cell)	Size2 (For 2×10^7 cell)
Volume	1mL	200μl
Bead Concentration	1×10^7 beads/mL	
Storage Buffer	PBS containing 0.1% BSA and 0.1% proclin-300, pH 7.4	

Storage Conditions and Shelf Life

Storage Conditions: Store at 2–8°C. DO NOT FREEZE.
Shelf Life: 2 years

Applications

This product is suitable for use with peripheral blood mononuclear cells (PBMC) or T cell subsets, including $CD3^+$ T cells, $CD4^+$ T cells, or $CD8^+$ T cells.

Reagents Required

Wash Buffer: PBS containing 2 mM EDTA and 2% fetal bovine serum (FBS) or PBS containing 2 mM EDTA and 0.5% BSA. Filter-sterilize through a 0.22 μm membrane before use.
Cell Culture Medium: RPMI 1640 supplemented with 2 mM glutamine and 10% FBS (optional: 100 U/mL penicillin-streptomycin). For T cell expansion, add 30 U/mL recombinant human IL-2 to the medium. Commercial T cell culture media are also suitable, and growth factor supplementation can be adjusted according to experimental requirements.

Important Notes

Thoroughly resuspend activation beads before use (e.g., vortex for >30 seconds or place on a rotator for 5 minutes). Avoid generating bubbles during pipetting. Before flow cytometry analysis, remove beads using a magnetic stand (including beads bound to cells; beads can be released by gentle

pipetting). The cells will be in the supernatant. Collect the supernatant for subsequent flow cytometry analysis.

Protocol

Bead Washing (Using Isolated Human CD3$^+$ T Cells as Example)

1. Thoroughly resuspend beads in the tube (e.g., vortex for >30 seconds or place on a rotator for 5 minutes).
2. Transfer the desired volume of beads to a flow tube and add an equal volume of Wash Buffer. If the total volume is less than 1 mL, add 1 mL of Wash Buffer, vortex for 5 seconds, or mix by pipetting. Avoid generating bubbles (cell culture medium may also be used for washing at this step).
3. Place the flow tube on a magnetic stand for 3 minutes and discard the supernatant.
4. Repeat Steps 2–3, this time using cell culture medium for the second wash. Wash beads a total of two times.
5. Resuspend beads in cell culture medium (e.g., if 25 µL of beads were taken for washing, resuspend them in 1 mL of cell culture medium after washing).

Human T Cell Activation (96-Well Plate Format)

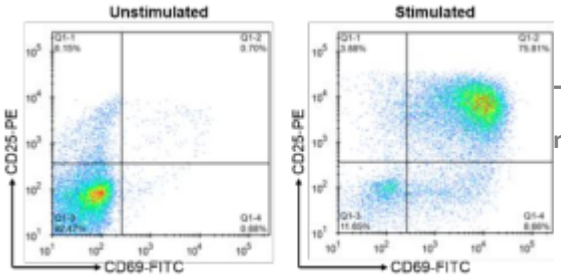
1. Adjust T cell concentration to 1×10^7 cells/mL. Add 25 µL of T cells per well (containing 2.5×10^5 T cells) and maintain a total culture volume of 100 µL.
2. Add 100 µL of washed and resuspended beads to each well for a 1:1 bead-to-cell ratio. (The bead-to-cell ratio can be adjusted according to experimental needs; a 1:1 ratio is recommended.)
3. Incubate cells and beads in a 37°C, 5% CO_2 incubator for the desired culture duration.
4. Harvest activated T cells for downstream experimental analysis.

Human T Cell Expansion

1. Seed CD3$^+$ T cells at a density of $1–1.5\times10^6$ cells/mL and add activation beads at a 1:1 bead-to-cell ratio.
2. Add 30 U/mL recombinant human IL-2 to the culture medium after 2 days of activation.
3. Incubate cells and beads in a 37°C, 5% CO_2 incubator for the desired culture duration.
4. Monitor cell activation and expansion daily, noting cell size and morphology. Cell shrinkage or significantly slowed proliferation indicates potential cell exhaustion.
5. Avoid handling cells for the first 2 days. After 2 days, monitor cell status continuously. Change medium or split cells when the medium turns yellow or cell density becomes too high. Replenish recombinant human IL-2 to 30 U/mL after each medium change or passage.
6. Count cells regularly. When cell density exceeds 2.5×10^6 cells/mL, gently pipette to mix and adjust cell density to $0.5–1\times10^6$ cells/mL.

Isolation Performance

Human CD3/CD28 T Cell Activation Beads were used to stimulate CD3$^+$ T cells for 24–48 hours. Cells were stained with FITC anti-human CD69 antibody (clone FN50) and PE anti-human CD25 antibody (clone BC96) and analyzed by flow cytometry. The percentage of CD25$^+$CD69$^+$ T cells was 0.70% pre-stimulation and 75.81% post-stimulation.



Important Notes

1. Avoid freezing magnetic beads and antibody mix during storage and use.
2. Use low-retention pipette tips and centrifuge tubes to minimize loss of beads and antibodies due to adsorption.
3. This product should be used with a magnetic separator.
4. For research use only.