Mouse CD3/CD28 T Cell Activation Beads

Catalog No.: BRC0012

Product Description

Mouse 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 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 mouse splenocytes 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 + 10% FBS + 50 µM β-mercaptoethanol (optional: 100 U/mL penicillin-streptomycin). For T cell expansion, add 30 U/mL recombinant Mouse 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 Mouse 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., 25 µL of beads can be used for 10 wells of T cell activation in a 96-well plate. After washing, resuspend in 1 mL of cell culture medium; add 100 µL of bead suspension per well for cell activation).

Mouse 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.

Mouse T Cell Expansion

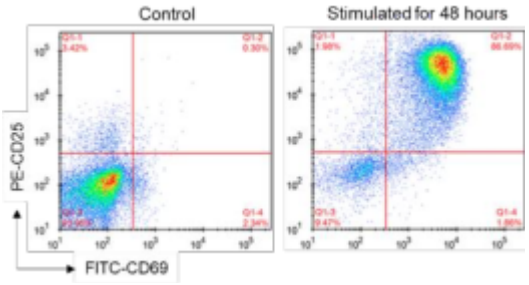
1. Activate T cells as described above. Assess T cell activation status at 48 hours post-bead addition. When cells are in good condition, gently pipette to split cells (if cell proliferation is slow, perform a half-medium change: carefully remove 100 µL of supernatant, add 100 µL of fresh culture medium, gently pipette to mix cells, continue culture for 1–2 days, then split). Finally, add 30 U/mL recombinant IL-2 to the well.
2. Culture cells and beads in a 37°C, 5% CO_2 incubator for the desired duration.
3. Monitor cell expansion daily or every other day. Mouse T cell expansion is characterized by visible proliferative clustering under microscopy. Normally, T cells exhibit rapid proliferation between days 4–12 post-activation. Cell shrinkage or significantly slowed proliferation indicates potential cell exhaustion.

Note: Do not handle cells or add recombinant IL-2 within the first 48 hours of activation. After 2 days, monitor cell status continuously. Change medium or split cells when the medium turns yellow or cell density becomes too high, and add recombinant IL-2. Replenish recombinant IL-2 to 30 U/mL after each medium change or passage. (Regular cell counting is recommended. When cell density exceeds 2.5×10^6 cells/mL, gently pipette to mix and adjust cell density to 0.5–1×10^6 cells/mL.)

Activation Performance

Mouse CD3/CD28 T Cell Activation Beads were used to stimulate CD3$^+$ T cells for 24–48 hours. Cells

were stained with FITC anti-mouse CD69 antibody (clone H1.2F3) and PE anti-mouse CD25 antibody (clone PC61) and analyzed by flow cytometry. The percentage of $CD25^{+}CD69^{+}$ T cells was 0.30% pre-stimulation and 86.69% 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.