

Mouse CD3/CD28 T Cell Activation Beads

Product SKU: AKES213



Product information:

Size: 0.2mL/1mL/5mL

Concentration: 1×10^8 beads/mL

Storage conditions: Store at 2 to 8°C. Do not freeze.

Introduction:

Two types of signals are required to induce T cell activation and proliferation. The first is a specific antigen-stimulating signal, generated by the binding of the TCR/CD3 complex to the MHC II antigen-peptide complex on the surface of antigen-presenting cells (APCs). The second is a non-specific co-stimulatory signal, produced by the interaction of co-stimulatory molecules on APCs with their corresponding receptors on T cells, with CD28 being one such co-stimulatory molecule.

Human CD3/CD28 T Cell Activation Beads are designed by coupling anti-CD3 and anti-CD28 antibodies to magnetic beads. These beads provide both the primary TCR signal and the necessary co-stimulatory signals for T-cell activation and expansion, thereby inducing T-cell activation and proliferation in vitro.

For subsequent experimental studies, the beads can be removed from cultured cells using a magnetic rack.

Required Materials (not included):

- RPMI-1640 complete medium (RPMI-1640 medium supplemented with penicillin solution at a final concentration of 100 U/mL and fetal bovine serum at a final concentration of 10%)
- Recombinant mouse IL-2
- Flat-bottom tissue culture plates of an appropriate size
- Magnet
- Mixer
- Humidified CO₂ incubator

Experimental protocol:

Sterile Procedure Requirements

All operations must be performed under sterile conditions.

1. Washing Mouse CD3/CD28 T Cell Activation Beads

- a) Before use, resuspend the beads in the vial to ensure a uniform suspension.
- b) Transfer the required amount of bead suspension into a 1.5 mL tube.
- c) Place the tube on a self-prepared 1.5 mL magnetic rack for 3 minutes and discard the supernatant while keeping the tube on the rack.
- d) Add an equal volume of RPMI-1640 complete medium and vortex for 10 seconds to mix.
- e) Repeat step (c).
- f) Add the same volume of RPMI-1640 complete medium as the original bead suspension, resuspend the beads by vortexing for 10 seconds, and set aside.

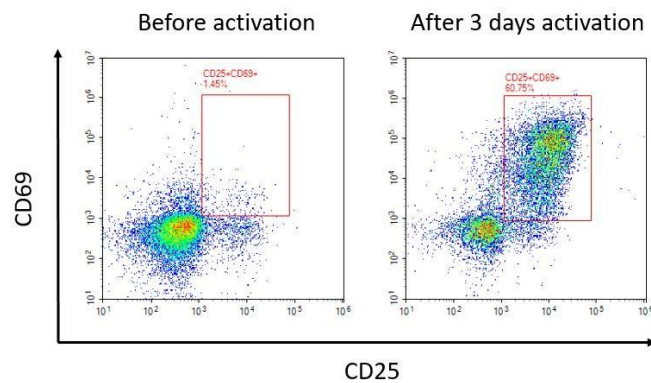
2. T Cell Activation

- a) Start with 1×10^6 purified T cells in 1 mL medium in a tissue culture plate.
- b) Add 10 μ L of beads per 1×10^6 T cells (1:1 ratio of beads to T cells) and mix by gentle pipetting.
- c) Incubate in a humidified CO₂ incubator at 37°C.
- d) Harvest the activated T cells according to your specific experimental requirements.
- e) For flow cytometry applications, remove the beads before staining:
 - Place the tube containing the cells on a magnetic rack for 3 minutes.
 - Collect the supernatant containing the cells for testing.

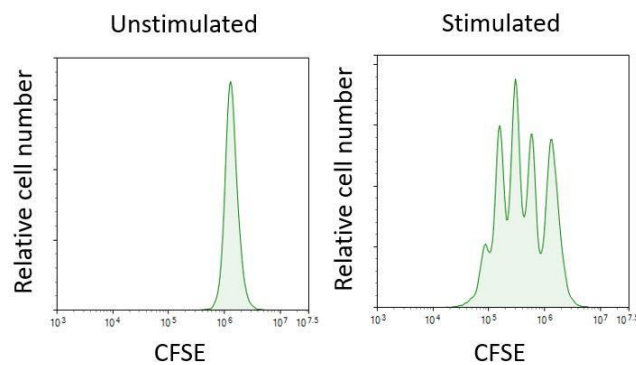
3. T Cell Expansion

- a) Start with 1×10^6 purified T cells in 1 mL medium in a tissue culture plate.
- b) Add 10 μ L of beads per 1×10^6 T cells (1:1 ratio of beads to T cells) and 8 ng/mL IL-2, then mix by gentle pipetting.
- c) Incubate in a humidified CO₂ incubator at 37°C.
- d) Count cells every two days. If the cell density exceeds 2.5×10^6 cells/mL or if the medium turns yellow, dilute the culture back to $0.5\text{--}1 \times 10^6$ cells/mL using culture medium containing rIL-2.

Typical results:



CD25 and CD69 expression in T cells was analyzed before activation and two days after activation.



CFSE-stained T cells were cultured for 3 days. The results were compared between the inactivated and activated groups.

Precautions:

- This product is for scientific use only.
- For your safety and health, please wear laboratory coats and disposable gloves during operation, and follow standard laboratory reagent handling procedures.
- Before using the beads, thoroughly vortex and mix them. Avoid air bubbles during the aspiration process to ensure consistent bead amounts and accurate experimental results.
- Place the tubes on a magnetic rack for at least 3 minutes to prevent bead loss.
- Carefully and slowly remove the beads after magnetic separation to avoid bead loss.

-For beads volumes less than or equal to 0.1 mL, wash with 0.1 mL of medium; for volumes greater than 0.1 mL, use an equal volume of medium.

-After removing the beads from magnetic separation, promptly add liquid resuspension to prevent the beads from drying out, which could affect their performance.

-If cell activation is poor or cell death is high, increase the bead amount; if necessary, decrease the bead amount when conditions improve.

-For flow cytometry applications, remove the beads prior to staining.

-Do not freeze the beads.

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If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details



Email: info@assaygenie.com

Web: www.assaygenie.com