Mouse CD3/CD28 T Cell Activation Beads

Cat. No: MIM001A **Size: 0.2mL/1mL/5mL**

Cat.	Products	Concentration	Size			Storage
MIM001A	Mouse CD3/CD28 T Cell Activation Beads	1×10^8beads/mL	0.2 mL	1 mL	1 mL×5	2-8°C
	Manual		One Copy			

Storage

Store at 2 to 8℃.Do not freeze。

Introduction

Two types of signals are required to induce T cell activation and proliferation: one is a specific antigen-stimulating signal generated by the binding of TCR/CD3 complex to the specific MHC II antigen-peptide complex on the surface of antigen-presenting cells (APCs); the other is a non-specific co-stimulatory signal generated by the interaction of co-stimulatory molecules on the surface of APCs with the corresponding receptors on the T cells, of which CD28 is one of the co-stimulatory molecule. Mouse CD3/CD28 T Cell Activation Beads are made by coupling anti-CD3 and anti-CD28 antibodies to magnetic beads, which can provide the primary TCR signal and co-stimulatory signals required for T-cell activation and expansion, thus inducing T-cell activation and proliferation *in vitro*. For subsequent relevant experimental studies, this beads can be removed from cultured cells by using a magnetic rack.

Materials Not Supplied

- **Reagents：**

  RPMI-1640 complete medium (penicillin solution (final concentration of 100U/mL), fetal bovine serum (final concentration of 10%) to RPMI 1640 medium), Recombinant mouse IL-2

- **Consumable**

  Flat bottom tissue culture plates of suitable size

- **Instruments**

  Magnet, Mixer, Humidified CO_2 incubator

Experimental Protocol

The following operations need to be performed under sterile conditions

➢ **Wash Mouse CD3/CD28 T Cell Activation Beads**

a) Before use, resuspend the beads in the vial to obtain a uniform suspension.

b) Take the required amount of beads suspension into a 1.5mL tube.

c) Place the tube on a 1.5 mL magnetic rack (self-prepared) for 3 minutes and discard the supernatant (while the tube is on the magnetic rack).

d) Add an equal volume RPMI-1640 complete medium, and vortex for 10 seconds to mix.

e) Place the tube on a 1.5 mL magnetic rack (self-prepared) for 3 minutes and discard the supernatant (while the tube is on the magnetic rack).

f) Add the same volume of RPMI-1640 complete medium as the beads suspension, resuspend the beads by vortexing for 10 seconds, and set aside.

- **Activate T cells**

a) Start with 1×10^6 purified T cells in 1mL medium in a tissue cultureplate.

b) Add 10 μL of beads per 1×10^6 T cells, namely a 1:1 ratio of beads to T cells, and mix by blowing.

c) Incubate in a humidified CO_2 incubator at 37°C.

d) According to your specific experimental requirements, harvest the activated T cells.

e) For flow cytometry applications, remove the beads prior to staining. Place the tube containing the cells on a magnetic rack for 3 minutes and the supernatant containing the cells was collected for testing.

- **Expand T cells**

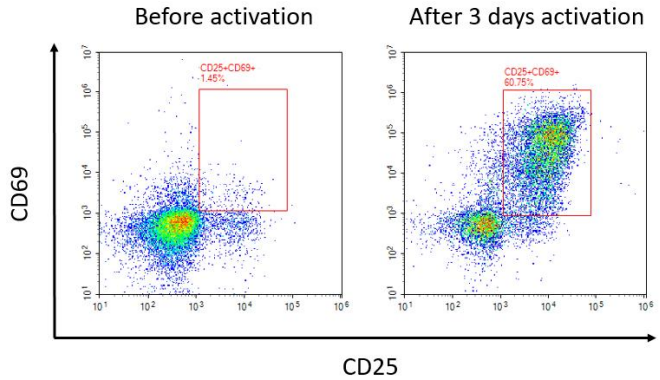
a) Start with 1×10^6 purified T cells in 1mL medium in a tissue cultureplate.

b) Add 10 μL of beads per 1×10^6 T cells, namely a 1:1 ratio of beads to T cells and 8 ng/mL rIL-2, then mix by blowing.

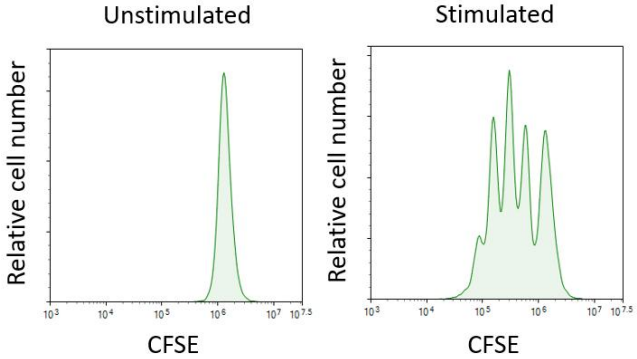
c) Incubate in a humidified CO_2 incubator at 37°C.

d) Cells were counted every two days.When the cell density exceeds 2.5×10^6 cells/mL or when the medium turns yellow, split cultures back to a density of $0.5\text{-}1\times10^6$ cells/mL in culture medium containing rIL-2.

Typical results



CD25 and CD69 expression of T cells were detected before activation and 2 days after activation



CFSE-stained T cells were cultured for 3 days,the result of T cells in the inactivated and activated groups

Cautions

1. This product is for scientific use only.
2. For your safety and health, please wear laboratory overalls and disposable gloves for operation, and follow the laboratory reagent operating procedures.
3. Before using the beads, they should be thoroughly vortex and mixed. Air bubbles should be avoided during the aspiration process, so as to avoid the difference in the amount of beads affecting the results of the subsequent experiments.
4. Tubes on a magnetic rack for not less than 3 minutes to avoid beads loss.
5. Please careful and slow removal of the beads after magnetic suction to avoid loss of beads.
6. It is recommended to wash with 0.1 mL for beads volume less than 0.1 mL or equal volume for volume more than 0.1 mL.
7. After removing the beads from the magnetic separation, liquid resuspension should be added as soon as possible to prevent the beads from drying out and affecting the use of the effect.
8. Beads can be increased appropriately, when the cell activation effect is poor, and when there is more cell death, the amount of magnetic beads can be reduced appropriately.
9. For flow cytometry applications, remove the beads prior to staining.
10. Do not freeze the beads.