# Human CD3/CD28 T Cell Activation Beads

**Product SKU:** AKES212

**Product information:**

**Size:** 0.2mL/1mL/5mL

**Concentration**: $1\times10^8$beads/mL

**Storage conditions:** Store at 2 to 8℃. 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 human IL-2
-Flat-bottom tissue culture plates of an appropriate size
-Magnet
-Mixer
-Humidified $CO_2$ incubator

## Experimental protocol:

### Sterile Procedure Requirements

All operations must be performed under sterile conditions.

### 1. Washing Human 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

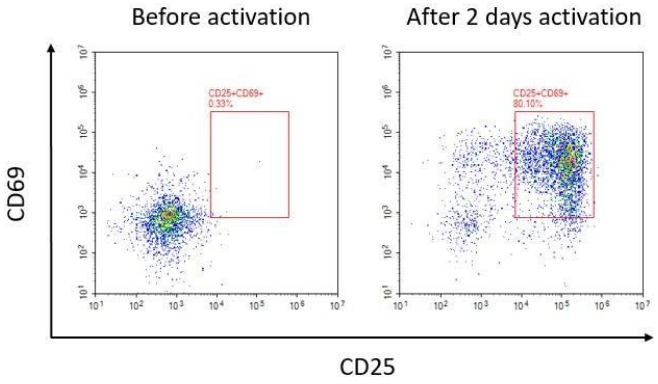
a) Start with $1 \times 10^6$ purified T cells in 1 mL medium in a tissue culture plate.
b) Add 10 µL of beads per $1 \times 10^6$ T cells (1:1 ratio of beads to T cells) and mix by gentle pipetting.
c) Incubate in a humidified $CO_2$ incubator at 37°C.
d) Harvest the activated T cells according to your 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.

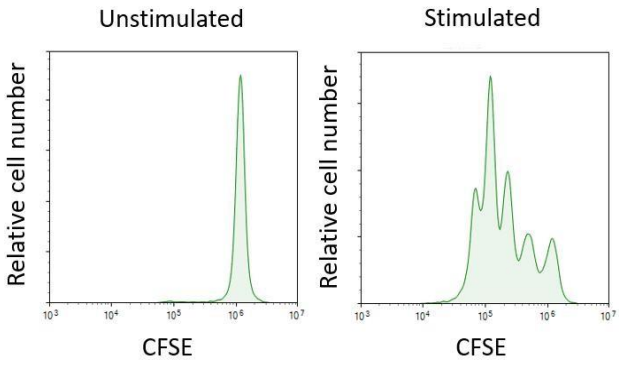
### 3. T Cell Expansion

a) Start with $1 \times 10^6$ purified T cells in 1 mL medium in a tissue culture plate.
b) Add 10 µL of beads per $1 \times 10^6$ 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 $CO_2$ incubator at 37°C.
d) Count cells every two days. If the cell density exceeds $2.5 \times 10^6$ cells/mL or if the medium turns yellow, dilute the culture back to $0.5–1 \times 10^6$ 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.

**Assay Genie 100% money-back guarantee!**

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

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