Human CD3/CD28 T Cell Activation Beads Kit

Kit Contents (Cat #:KMS310)

- Functional Grade Biotin-CD3(OKT3)(Cat #:**MS310A**)
- Functional Grade Biotin-CD28(28.2)(Cat #:**MS310B**)
- Cell culture Grade Streptavidin Magnetic Beads (Cat #:**MS310C**)

Background

T lymphocytes are activated through MHC class II molecules on antigen-presenting cells (APC). After activation, T cells expand rapidly and secrete various cytokines. The Human CD3/CD28 T cell Activation Beads Kit is designed to activate and expand human T cells. This Kit contains Biotinylated anti-human CD3 and CD28 antibodies, as well as cell culture grade Streptavidin magnetic beads. After Streptavidin magnetic beads are loaded with biotinylated CD3/CD28, they mimic antigen presenting cells and can activate resting T lymphocytes from human PBMCs or purified T lymphocytes. After 2-3 days of activation, magnetic beads can easily be removed by magnet. Further T lymphocytes expansion will require human cytokines for *in vitro* culture.

Supplies and Reagents

1. Incubation Buffer: PBS and 0.5% HSA, 2mM EDTA, pH7.4 (PBS should be Ca^{2+} or Mg^{2+} free)
2. Cell culture media: RPMI-1640 + 10% FBS or other T cell expansion media
3. Cell culture plate or flask
4. Rotator in 4°C
5. Magnet
6. Sterile tubes
7. Human recombinant IL-2 (HZ-1050) or other cytokines for T cell expansion

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Protocol

1. Sample Preparation

Human CD3/CD28 T cell Activation Beads Kit is designed to activate human T cells from total PBMC or after cell sorting. For whole blood or Leukopak samples, PBMC should be isolated by a gradient centrifugation method, such as Ficoll-Paque. For T cells obtained through cell sorting, such as CD4+ cells or CD8+ cells, follow user's manual to obtain desired T cell population before activation.

2. Loading of CD3/CD28 Magnetic Beads

a) Pipette 100µL Biotin-CD3, 100µL Biotin-CD28 antibody, and 300µL Incubation Buffer (PBS and 0.5% HSA, 2mM EDTA, pH7.4) in a sterile 2mL tube and mix well.

b) Resuspend Streptavidin Magnetic beads (MS310C-10 or MS310C-100) and vortex well.

c) Add 500µL Streptavidin Magnetic Beads to the Biotin antibody mix. Gently rotate the tube on a rotator in 2-8°C for 2 hours.

d) The loaded CD3/CD28 beads are ready to use and can be stored in 2-8°C for up to 6 months.

3. T cell Activation

a) Resuspend loaded CD3/CD28 magnetic beads, and transfer 100 µL to a separate tube (100µL loaded beads per $1x10^7$ human PBMCs or isolated T cells).

b) Wash beads by adding 400µL cell culture media to loaded CD3/C28 beads and placing on magnet for 2 minutes.

c) Remove the supernatant, then remove tube from magnet and resuspend beads with 100µL cell culture media.

d) Add the washed CD3/CD28 beads at a 1:1 ratio of beads to cells. Each 10µL loaded CD3/CD28 beads can be used to activate $1x10^6$ cells in 1ml cell culture media. Scale up beads and all other reagents accordingly for larger scale T cell activation. For suggested bead usage for different cell numbers, please refer to Table 1.

Protocol

Table 1. Scaling of CD3/CD28 beads activation

	per well in 6 well plate	per well in 12 well plate	per well in 24 well plate	per well in 48 well plate	per well in 96 well plate
Cell number	$1x10^7$	$5x10^6$	$2.5x10^6$	$1.25x10^6$	$5x10^5$
Loaded CD3/CD28 bead volume	100µL	50µL	25µL	12.5µL	5µL
Cell culture media volume	2ml	1ml	0.5ml	0.25ml	0.1ml

e) Place the cell culture plate in 37°C, 5% CO_2 for up to 3 days. (Note: for different experimental purposes, activation time should be optimized. Overactivation of T cells will lead to cell death. Inspect cell culture media daily, add fresh media if necessary.)

f) After 3 days of activation, activated T cells can be collected for analysis such as flow cytometry or immunofluorescence staining. To remove beads from cell culture media, transfer cells to a tube and place on magnet for 5 minutes. Collect the supernatant containing the activated T cells to a new tube. Discard beads.

4. T cell Expansion

a) Perform T cell activation as described in previous steps for 3 days.

b) At day 3, move CD3/CD28 beads and adjust cell density to $1x10^6$/mL, add 30U/mL human recombinant IL-2 (HZ-1015) to cell culture media and place cell culture plates or flasks in 37°C, 5% CO_2 for culturing.

c) Inspect cell culture media every day and add fresh media containing human recombinant IL-2 when needed. Count cell numbers at least twice a week.

d) After 14 days of culture, 100- to 1000-fold expansion of T cells can be achieved for downstream applications. For additional T cell expansion, perform T cell Activation steps again using CD3/CD28 beads for re-stimulation.