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Mouse CD3/CD28 T Cell Activation Beads Kit

KIT CONTENTS (Cat No. KMS311)

- Functional Grade Biotin-CD3 (145-2C11) **(Cat No. MS311A)**
- Functional Grade Biotin-CD28 (37.51.1) **(Cat No. MS311B)**
- Cell culture Grade Streptavidin Magnetic Beads **(Cat No. MS311C)**

Details

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 Mouse CD3/CD28 T Cell Activation Beads Kit is designed to activate and expand mouse T cells. This kit contains biotinylated anti-mouse 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 mouse splenocytes or purified T lymphocytes. After 2-3 days of activation, beads can easily be removed by magnet. Further T lymphocytes expansion may require specific additives for in vitro culture. After 7-10 days in culture, cells can be restimulated by repeating the protocol.

Supplies and Reagents

- **Incubation Buffer:**
  PBS with 0.5% serum albumin (HSA or BSA) and 2mM EDTA, pH7.4; sterile filtered (PBS should be Ca2+ and Mg2+ free)
- **Cell culture media: RPMI-1640 + 10% FBS + 1% Penicillin/streptomycin + 55μm 2-mercaptoethanol (or other T cell media)**
- **Cell culture plate or flask**
- **Rotator in 2-8°C**
- **Magnet**
- **Sterile tubes**

Protocol

Loading of CD3/CD28 Magnetic Beads

1. Combine Biotin-CD3 and Biotin-CD28 antibodies, and Incubation Buffer in a sterile tube and mix well. See **Table 1** for recommended amounts.
2. Resuspend Streptavidin Magnetic beads (MS311C-10 or MS311C-100) by vortexing for at least 20 seconds.
3. Add Streptavidin Magnetic Beads to the Biotin antibody mix. See **Table 1** below for recommended amount.
4. Gently rotate the tube on a rotator in 2-8°C for 2 hours.
5. Final concentration of loaded CD3/CD28 beads is $1x10^8$ beads per ml. The recommended ratio for activation is 1:1 beads to cells.

Table 1. Scaling of Components for Loading

Number of cells for activation	Recommended bead loading amounts		
	Biotin antibodies	Incubation buffer	Streptavidin beads
$1x10^7$	10μl each	30μl	50μl
$1x10^8$	100μl each	300μl	500μl
$1x10^9$	1ml each	3ml	5ml

General Guidelines

- It is not recommended to load fewer than 1 test ($1x10^7$ cells) at a time.
- All calculations are for a 1:1 beads to cells ratio.
- Loaded CD3/CD28 beads can be stored in 2-8°C for up to 6 months.

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Protocol (continued)

Sample Preparation

The Mouse CD3/CD28 T Cell Activation Beads Kit is designed to activate mouse T cells from total splenocytes or isolated T cells after cell sorting. Resuspend cells to 1x10^6/ml in RPMI-1640 + 10% FBS + 1% Penicillin/streptomycin + 55μm 2-mercaptoethanol or other T cell media.

T Cell Activation

1. Follow protocol for "Loading of CD3/CD28 Magnetic Beads."
2. Vortex loaded CD3/CD28 magnetic beads, and transfer desired amount of beads to a tube suitable for magnetic separation. See **Table 2** for recommended amounts.
3. Wash beads by adding an equal volume or at least 1ml of cell culture media to loaded CD3/CD28 beads. Mix well and place on magnet for 2 minutes.
4. Remove the supernatant without disturbing the beads, then remove tube from magnet and resuspend beads in cell culture media to the same volume as the initial volume of beads used in step 2.
5. Add the washed CD3/CD28 beads to resuspended cells. Each 10μL loaded CD3/CD28 beads can be used to activate 1x10^6 cells.
6. Culture cells at 37°C with 5% CO_2 for 2-3 days.
7. After 2-3 days of activation, remove beads from cells by mixing with a pipette then transferring to a tube. Place tube on magnet for 5 minutes.
8. Collect the supernatant containing the activated T cells to a new tube. Discard beads. Activated T cells can then be used for analysis such as flow cytometry or immunofluorescence staining or can be cultured further. After 7-10 days of culture, the cells can be restimulated by repeating the protocol.

Note: For different experimental purposes, the activation time, cell density, and bead to cell ratio should be optimized. Over-activation of T cells will lead to cell death. Inspect cell culture media daily, add fresh media and cytokines as necessary.

Table 2. Scaling of CD3/CD28 Beads for Activation

Number of cells for activation	Recommended activation set up*	
	Culture volume	Loaded beads volume
1x10^5	100μl	1μl
1x10^6	1ml	10μl
1x10^7	10ml	100μl
1x10^8	100ml	1ml

**For cells at a concentration of 1x10^6/ml.*

Related Products

Mouse Activated T Cell Panel

Anti-Mouse Flow Cytometry Antibodies

Mouse Cytokine ELISA kits

Magnetic Cell Separation Kits