ImmunoCult™ Mouse T Cell Activator Kit

Mouse T cell activation and expansion reagent

Catalog #100-1572 1 Kit

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

ImmunoCult™ Mouse T Cell Activator Kit is designed to activate and expand mouse T cells in the absence of magnetic beads, feeder cells, or antigens. ImmunoCult™ Mouse T Cell Activator Kit consists of soluble antibody complexes that bind CD3 and CD28 cell surface ligands, with an option to bind CD2. Binding of the antibody complexes results in the cross-linking of CD3 and CD28 cell surface ligands, thereby providing the required primary and co-stimulatory signals for T cell activation. Enhancement of T cell activation by the soluble antibody complexes may also be achieved by cross-linking of CD2. Activated mouse T cells can be expanded in different culture media (see section A), supplemented with cytokines.

- Robust activation and expansion of mouse T cells without the use of magnetic beads, feeder cells, or antigens
- Flexible mouse T cell activation by CD3/CD28 or CD3/CD28/CD2
- Supplied as a 100X concentrate that can be added directly to medium

Product Information

The following components are sold as a complete kit (Catalog #100-1572) and are not available for individual sale.

PRODUCT NAME	CATALOG #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
ImmunoCult™ Mouse T Cell Activator Component A	300-1032	1 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS and 0.1% BSA.
ImmunoCult™ Mouse T Cell Activator Component B	300-1030	1 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS and 0.1% BSA.
ImmunoCult™ Mouse T Cell Activator Component C	300-1040	1 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS and 0.1% BSA.

BSA - bovine serum albumin; PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

Preparation of Reagents and Materials

A. Mouse T Cell Growth Medium

Use a fully supplemented medium such as ImmunoCult™-XF T Cell Expansion Medium (Catalog #10981) as Mouse T Cell Growth Medium. It is recommended to add an antibiotic of choice (e.g. 50 µg/mL gentamicin).

Alternatively, use sterile technique to prepare Mouse T Cell Growth Medium by adding the following components to Iscove's Modified Dulbecco's Medium (IMDM) with 25 mM HEPES (Catalog #36150) and mix thoroughly:

- 10% fetal bovine serum
- 2 mM L-Glutamine (Catalog #07100)
- 50 µM ß-mercaptoethanol
- Preferred antibiotic (e.g. 50 µg/mL gentamicin)

NOTE: Other basal media such as RPMI 1640 Medium (Catalog #36750) or DMEM with 4500 mg/L D-Glucose (Catalog #36250), supplemented with 25 mM HEPES Buffer Solution (Catalog #07200), can also be used to prepare Mouse T Cell Growth Medium.

NOTE: If not used immediately, store Mouse T Cell Growth Medium at 2 - 8°C for up to 2 weeks.

B. Mouse T Cells

Isolate mouse T cells from freshly processed mouse splenocytes, using EasySep™ Mouse T Cell Isolation Kit (Catalog #19851). ImmunoCult™ Mouse T Cell Activator Kit is also compatible with other mouse T cell isolation kits such as:

- EasySep™ Mouse CD4+ T Cell Isolation Kit (Catalog #19852)
- EasySep™ Mouse CD8+ T Cell Isolation Kit (Catalog #19853)
- EasySep™ Mouse Naïve CD4+ T Cell Isolation Kit (Catalog #19765)

Alternatively, mouse splenocytes depleted of red blood cells by Ammonium Chloride Solution (Catalog #07850) can be used. For complete instructions, refer to the Product Information Sheet #10000000361-PIS_01, available at stemcell.com, or contact us to request a copy.

C. Mouse T Cell Expansion Medium and Mouse T Cell Activators

1. Prepare fresh Mouse T Cell Expansion Medium by adding cytokines (e.g. 30 U/mL Mouse Recombinant IL-2; Catalog #78081) to the Mouse T Cell Growth Medium (prepared in section A). Mix thoroughly.

   NOTE: Mouse T Cell Expansion Medium must be prepared fresh on each day of use.

2. To prepare Mouse CD3/CD28 T Cell Activator, add the following components to fresh Mouse T Cell Expansion Medium (prepared in step 1):

   a. Add ImmunoCult™ Mouse T Cell Activator Component A at a 1 in 100 dilution. Mix thoroughly.

   b. Add ImmunoCult™ Mouse T Cell Activator Component C at a 1 in 100 dilution. Mix thoroughly.

   OR

   To prepare Mouse CD3/CD28/CD2 T Cell Activator, add the following components to fresh Mouse T Cell Expansion Medium (prepared in step 1):

   a. Add ImmunoCult™ Mouse T Cell Activator Component A at a 1 in 100 dilution. Mix thoroughly.

   b. Add ImmunoCult™ Mouse T Cell Activator Component B at a 1 in 100 dilution. Mix thoroughly.

   c. Add ImmunoCult™ Mouse T Cell Activator Component C at a 1 in 100 dilution. Mix thoroughly.

   NOTE: Once supplemented with ImmunoCult™ Mouse T Cell Activator complexes, the medium must be used within the same day of preparation.

Directions for Use

Please read the entire protocol before proceeding. This is a general protocol for using ImmunoCult™ Mouse T Cell Activator Kit. Depending on the experimental objectives, optimization may be required (e.g. cell seeding density and cytokine concentration) for optimal growth.

1. On day 0:

   a. Isolate mouse T cells (see section B).

   b. Prepare fresh Mouse T Cell Expansion Medium (see section C, step 1).

   c. Prepare Mouse CD3/CD28 or CD3/CD28/CD2 T Cell Activator (see section C, step 2).

   d. Seed viable mouse T cells (prepared in section B) at 1 x 10^6 cells/mL in Mouse T Cell Expansion Medium supplemented with Mouse T Cell Activator (prepared in section C, step 2). Recommended culture volumes can be found in Table 1.

   e. Incubate at 37°C and 5% CO_2 in a humidified incubator for up to 3 days.

Table 1. Number of Cells and Volume of Cell Suspension Recommended for Various Cultureware

CULTUREWARE	TOTAL NUMBER OF CELLS/WELL	VOLUME OF CELL SUSPENSION/WELL
6-well plate	2 - 4 x 10^6	2 - 4 mL
12-well plate	1 - 2 x 10^6	1 - 2 mL
24-well plate	0.5 - 1 x 10^6	0.5 - 1 mL
48-well plate	2.5 - 5 x 10^5	0.25 - 0.5 mL
96-well plate	1 - 2 x 10^5	0.1 - 0.2 mL

2. To expand T cells after 2 - 3 days of activation, mix the cell suspension thoroughly and perform a viable cell count. **Maintain the viable cell density below 1 x 10^6 cells/mL** by adding fresh Mouse T Cell Expansion Medium to the cell suspension every 2 - 3 days. An example procedure for culturing mouse T cells (starting after 2 - 3 days of activation) can be found below:
   - Day 2 - 3: Mix the cell suspension thoroughly, perform a viable cell count, and increase the volume of the cell suspension 8-fold (adjust the viable cell density to ~1 - 2.5 x 10^5 cells/mL) by adding fresh Mouse T Cell Expansion Medium. Incubate at 37°C and 5% CO_2 for 2 - 3 days.
   - Day 5 - 6: Mix the cell suspension thoroughly, perform a viable cell count, and increase the volume of the cell suspension 4-fold (adjust the viable cell density to ~1 - 3 x 10^5 cells/mL) by adding fresh Mouse T Cell Expansion Medium. Incubate at 37°C and 5% CO_2 for 2 - 3 days.
   - Day 7 - 8: Harvest cells if the desired cell number is achieved.

   NOTE: Additional Mouse T Cell Activator reagent is not required throughout the 7- to 8-day culture period.

   NOTE: Ensure fresh Mouse T Cell Expansion Medium is added every 2 - 3 days; do not wait more than 3 days between medium additions.

Data

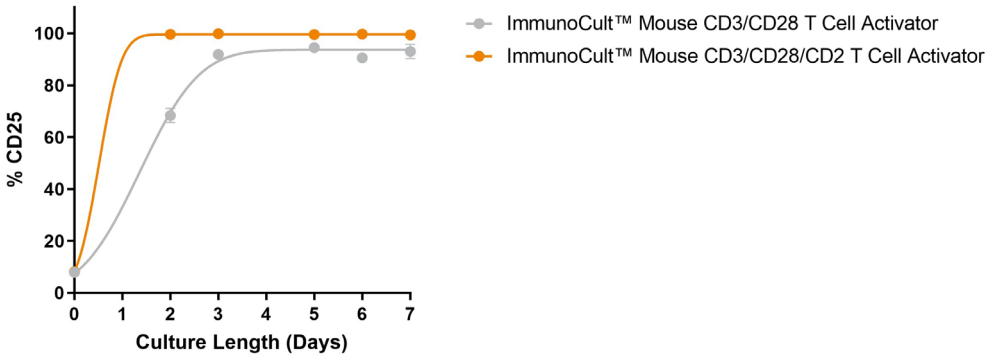

Figure 1. Activation of EasySep™-Isolated Mouse T Cells Stimulated with ImmunoCult™ Mouse T Cell Activator Kit

Mouse T cells were isolated using EasySep™ Mouse T Cell Isolation Kit, stimulated with ImmunoCult™ Mouse T Cell Activator Kit, and cultured in Mouse T Cell Expansion Medium. Following 3 days of culture, the mean ± SD frequency of CD25+ cells was 91.9 ± 5.1% (n = 11) or 99.9 ± 0.1% (n = 5), when stimulated with ImmunoCult™ Mouse CD3/CD28 T Cell Activator or ImmunoCult™ Mouse CD3/CD28/CD2 T Cell Activator, respectively. Stimulated mouse T cells maintained expression levels of CD25 throughout the 7-day culture period.

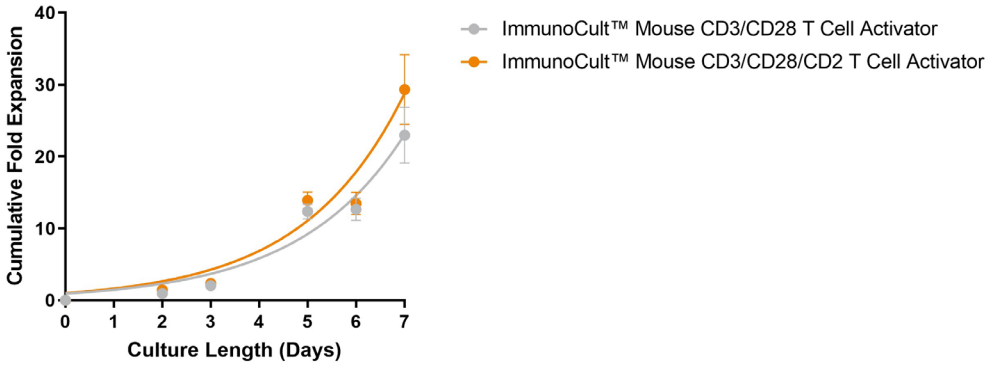

Figure 2. Expansion of EasySep™-Isolated Mouse T Cells Stimulated with ImmunoCult™ Mouse T Cell Activator Kit

EasySep™-isolated mouse T cells were expanded with ImmunoCult™ Mouse T Cell Activator Kit in Mouse T Cell Expansion Medium over 7 days. The number of viable cells was assessed every 2 - 3 days, and fresh medium supplemented with IL-2 was added. No additional ImmunoCult™ Mouse T Cell Activator was added during the 7-day culture period. After 7 days in culture with ImmunoCult™ Mouse CD3/CD28 T Cell Activator or ImmunoCult™ Mouse CD3/CD28/CD2 T Cell Activator, stimulation resulted in a fold expansion of 23 ± 3.4 or 29.3 ± 4.8 (mean ± SEM, n = 6), respectively.

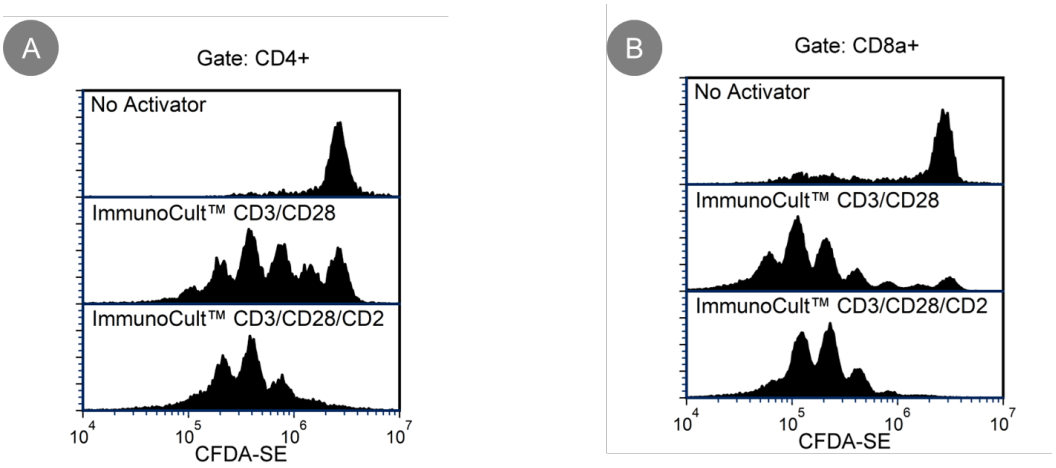

Figure 3. Cell Proliferation of EasySep™-Isolated T cells Stimulated with ImmunoCult™ Mouse T Cell Activator Kit

EasySep™-isolated mouse T cells were labeled with CFDA-SE (Catalog #75003), stimulated with ImmunoCult™ Mouse T Cell Activator Kit, and cultured in Mouse T Cell Expansion Medium. On day 3, cells were harvested, stained with anti-mouse CD4 and CD8a antibodies, then measured by flow cytometry. Shown are CFDA-SE-labeled mouse T cells, gated on viable CD4+ (A) or CD8a+ (B) cells, cultured with no activator (top panel), with ImmunoCult™ Mouse CD3/CD28 T Cell Activator (middle panel), or with ImmunoCult™ Mouse CD3/CD28/CD2 T Cell Activator (bottom panel). Due to cell proliferation, the intensity of CFDA-SE signal is reduced by 50% for each cell division.

Notes and Tips

ANTIGEN DETECTION

ImmunoCult™ Mouse T Cell Activator Kit contains an anti-CD3 antibody clone that, to our knowledge, fully or partially blocks all anti-CD3 antibody clones used to assess CD3 expression by flow cytometry. For detection of activated T cells by flow cytometry, use alternative markers such as fluorochrome-conjugated Anti-Mouse CD4 Antibody, Clone RM4-5 (Catalog #60017) and Anti-Mouse CD8a Antibody, Clone 53-6.7 (Catalog #60023).

Related Products

For related products, including specialized culture and storage media, supplements, antibodies, cytokines, and small molecules, visit www.stemcell.com, or contact us at techsupport@stemcell.com.