

# ImmunoCult™ Human CD3/CD28 T Cell Activator



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Catalog #10971      2 mL  
#10991              5 x 2 mL

## Product Description

ImmunoCult™ Human CD3/CD28 T Cell Activator is designed to activate and expand human T cells in the absence of magnetic beads, feeder cells, or antigen. ImmunoCult™ Human CD3/CD28 T Cell Activator consists of soluble antibody complexes that bind CD3 and CD28 cell surface ligands. 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. Activated T cells can be expanded in ImmunoCult™-XF T Cell Expansion Medium (Catalog #10981) or other media for culturing human T cells supplemented with cytokines.

- Robust activation and expansion of human T cells without the use of magnetic beads, feeder cells, or antigen
- Provides a gentle activation stimulus that maintains high viability of activated and expanded T cells
- Highly stable, filter-sterilized soluble reagent

## Properties

**Storage:** Store at 2 - 8°C.

**Shelf Life:** Stable until expiry date (EXP) on label.

**Contains:**

- Anti-human CD3 monospecific antibody complex
- Anti-human CD28 monospecific antibody complex

## Directions for Use

NOTE: This is a general protocol for using ImmunoCult™ Human CD3/CD28 T Cell Activator. Depending on the experimental objectives, optimization may be required (e.g. cell seeding density and cytokine concentration) for optimal cell growth.

1. Isolate human T cells from fresh or previously frozen peripheral blood mononuclear cells, or leukapheresis samples, using one of the following EasySep™ kits:
  - EasySep™ Release Human CD3 Positive Selection Kit (Catalog #17751)
  - EasySep™ Human T Cell Enrichment Kit (Catalog #19051)
  - EasySep™ Human T Cell Isolation Kit (Catalog #17951)

NOTE: Isolated T cells can be cryopreserved using CryoStor® CS5 (Catalog #07933) or CryoStor® CS10 (Catalog #07930) and stored at -135°C.

2. On day 0:
  - a. Prepare fresh complete ImmunoCult™-XF T Cell Expansion Medium by adding cytokines (e.g. Human Recombinant IL-2; Catalog #78036) to ImmunoCult™-XF T Cell Expansion Medium. Mix thoroughly.  
NOTE: Complete ImmunoCult™-XF T Cell Expansion Medium must be prepared fresh on each day of use.
  - b. Seed viable human T cells (prepared in step 1) in fresh complete ImmunoCult™-XF T Cell Expansion Medium (prepared in step 2a) at  $1 \times 10^6$  cells/mL.
3. To activate T cells, add 25  $\mu$ L/mL of ImmunoCult™ Human CD3/CD28 T Cell Activator to the cell suspension. Incubate cells at 37°C and 5% CO<sub>2</sub> for up to 3 days.
4. To expand T cells (after 3 days of activation), perform a viable cell count and adjust the viable cell density every 2 - 3 days by adding fresh complete ImmunoCult™-XF T Cell Expansion Medium to the cell suspension.

NOTE: For recommended cell densities, refer to the Product Information Sheet for ImmunoCult™-XF T Cell Expansion Medium, available at [www.stemcell.com](http://www.stemcell.com) or contact us to request a copy.

5. Incubate cells at 37°C and 5% CO<sub>2</sub> until the desired cell number is obtained or for up to 12 days.
6. For longer-term expansion (> 12 days) of human T cells:
  - a. Harvest and resuspend the expanded T cells at  $1 \times 10^6$  cells/mL in fresh complete ImmunoCult™-XF T Cell Expansion Medium.
  - b. Restimulate by adding 25  $\mu$ L/mL of ImmunoCult™ Human CD3/CD28 T Cell Activator.

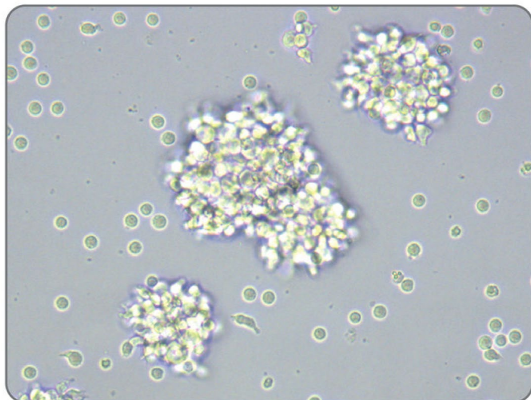
c. Incubate at 37°C and 5% CO<sub>2</sub>. Every 2 - 3 days, adjust cell density by adding fresh complete ImmunoCult™-XF T Cell Expansion Medium.

NOTE: Ensure to add fresh complete medium every 2 - 3 days; do not wait more than 3 days between medium additions.

## Related Products

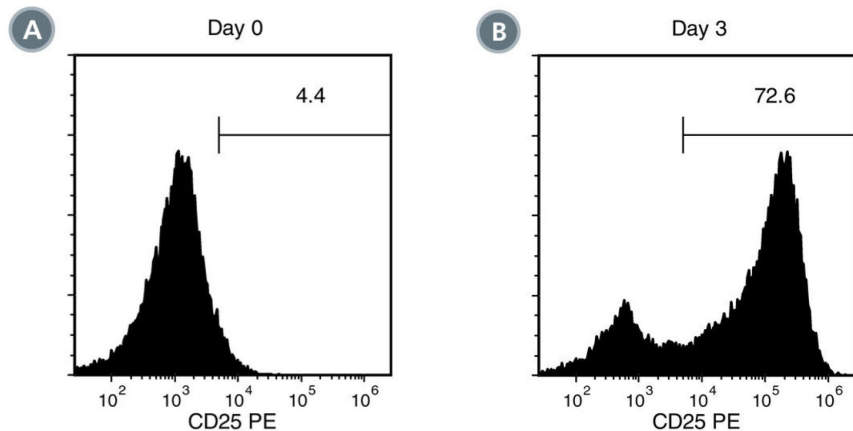
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## Data



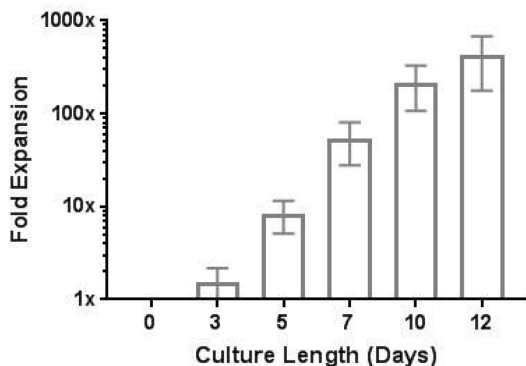
**FIGURE 1. Morphology of Activated Human T Cells Stimulated with ImmunoCult™ Human CD3/CD28 T Cell Activator**

Human T cells isolated using EasySep™ Human T Cell Isolation Kit (Catalog #17951), stimulated with ImmunoCult™ Human CD3/CD28 T Cell Activator, and cultured in complete ImmunoCult™-XF T Cell Expansion Medium.



**FIGURE 2. Activation of EasySep™-Isolated Human T Cells Stimulated with ImmunoCult™ Human CD3/CD28 T Cell Activator**

EasySep™-isolated human T cells were stimulated with ImmunoCult™ Human CD3/CD28 T Cell Activator and cultured in complete ImmunoCult™-XF T Cell Expansion Medium. Activation of viable CD3+ T cells was assessed by CD25 expression using flow cytometry. On day 0, the frequency of CD25-positive cells was (A)  $5.6 \pm 2.4\%$  (mean  $\pm$  SD). Following 3 days of culture, the frequency of CD25-positive cells was (B)  $75.4 \pm 13.8\%$  (mean  $\pm$  SD) when stimulated with ImmunoCult™ Human CD3/CD28 T Cell Activator.



**FIGURE 3. Robust Human T Cell Expansion with ImmunoCult™ Human CD3/CD28 T Cell Activator**

EasySep™-isolated human T cells were expanded over 12 days with ImmunoCult™ Human CD3/CD28 T Cell Activator in ImmunoCult™-XF T Cell Expansion Medium supplemented with Human Recombinant IL-2. On day 0,  $1 \times 10^6$  EasySep™-isolated human T cells were stimulated with 25  $\mu$ L of ImmunoCult™ Human CD3/CD28 T Cell Activator in ImmunoCult™-XF T Cell Expansion Medium supplemented with 10 ng/mL Human Recombinant IL-2. On days 3, 5, 7, and 10, viable cells were counted and fresh medium supplemented with IL-2 was added. No additional ImmunoCult™ Human CD3/CD28 T Cell Activator was added during the 12-day culture period (mean  $\pm$  SD in 6 experiments with 3 donors).

## Notes and Tips

- Activated T cells can be used for gene editing, usually 2 - 3 days after activation; the optimal activation period may vary with different gene editing approaches.

### ASSESSING PURITY

ImmunoCult™ Human CD3/CD28 T Cell Activator 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-Human CD4 Antibody, Clone OKT4 (Catalog #60016), Anti-Human CD8a Antibody, Clone RPA-T8 (Catalog #60022), and/or Anti-Human CD2 Antibody, Clone RPA-2.10 (Catalog #60007).

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