ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator

cGMP, Human T cell activation and expansion reagent

Catalog #100-0785 10 mL

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Product Description

ImmunoCult™ Human CD3/CD28/CD2 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/CD2 T Cell Activator consists of soluble antibody complexes that bind CD3, CD28, and CD2 cell surface ligands. Binding of the antibody complexes results in the cross-linking of CD3, CD28, and CD2 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 (Catalog #100-0956) or other serum-free 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

ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator is manufactured and tested following relevant cGMPs under a certified quality management system. For additional quality information, refer to www.stemcell.com/compliance.
NOTE: The product is intended for single use only.

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
- Anti-human CD2 monospecific antibody complex
- Phosphate buffered-saline (PBS), containing 0.02% TWEEN® 20

Directions for Use

NOTE: This is a general protocol for using ImmunoCult™ Human CD3/CD28/CD2 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 by adding cytokines (e.g. Human Recombinant IL-2 [Catalog #78036/78145]) to ImmunoCult™-XF. Mix thoroughly.

      NOTE: Complete ImmunoCult™-XF must be prepared fresh on each day of use.

   b. Seed viable human T cells (prepared in step 1) in fresh complete ImmunoCult™-XF (prepared in step 2a) at 1 x 10^6 cells/mL.
3. To activate T cells, add 25 µL/mL of ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator to the cell suspension. Incubate cells at 37°C and 5% CO_2 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 to the cell suspension.

   NOTE: For recommended cell densities, refer to the Product Information Sheet for ImmunoCult™-XF, available at www.stemcell.com, or contact us to request a copy.
5. Incubate cells at 37°C and 5% CO_2 until the desired cell number is obtained or for up to 12 days.

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

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Related Products

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

Data

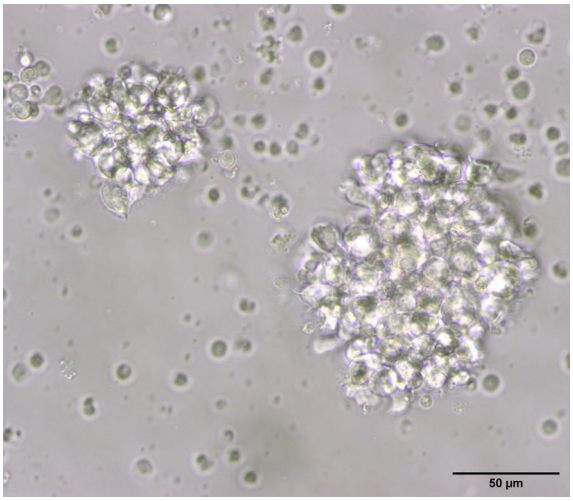

FIGURE 1. Morphology of Activated Human T Cells Stimulated with ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator
Human T cells isolated using EasySep™ Human T Cell Isolation Kit were stimulated with ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator for 3 days in complete ImmunoCult™-XF, supplemented with Human Recombinant IL-2.

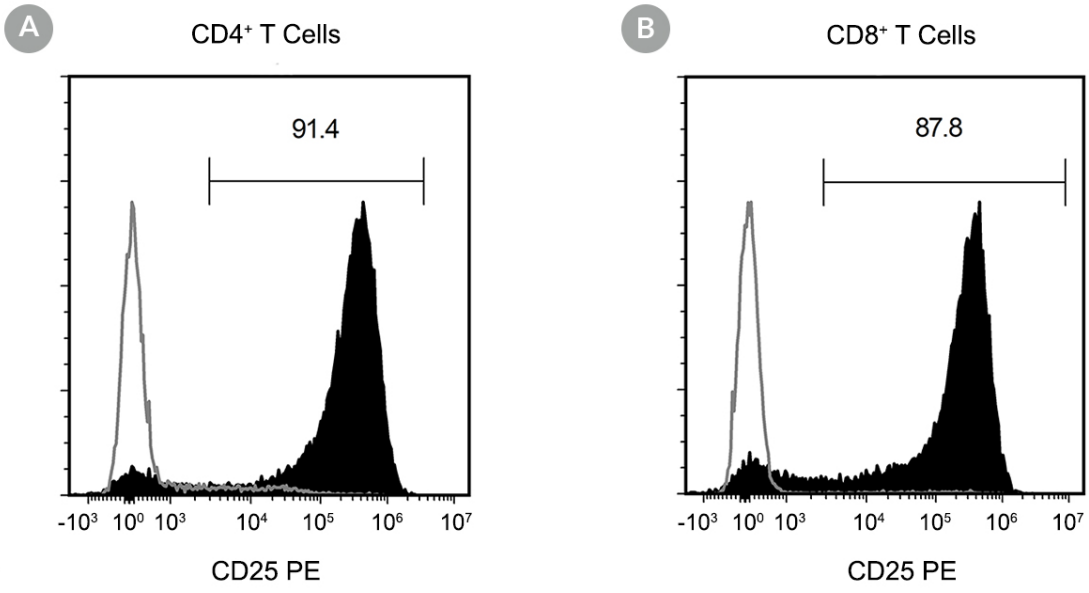

FIGURE 2. Activation of EasySep™-Isolated Human T Cells Stimulated with ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator
EasySep™-isolated human T cells were stimulated with ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator and cultured in complete ImmunoCult™-XF, supplemented with Human Recombinant IL-2. Activation of viable CD4+ and CD8+ T cells was assessed by CD25 expression, using flow cytometry. Following 3 days of culture, the frequency of CD25+ cells was **(A)** 91.4% for CD4+ T cells and **(B)** 87.8% for CD8+ T cells. The gray line depicts CD25 expression on CD4+ and CD8+ T cells after 3 days of culture without addition of ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator.

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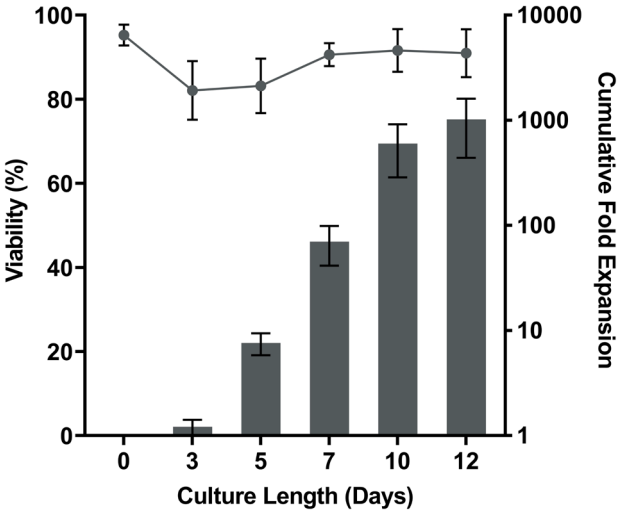

FIGURE 3. Robust Human T Cell Expansion and High Viability with ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator
On day 0, 1 x 10^6 EasySep™-isolated human T cells were stimulated with 25 µL of ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator in complete ImmunoCult™-XF, supplemented with 10 ng/mL Human Recombinant IL-2. On days 3, 5, 7, and 10, viable cells were counted, viability was measured, and fresh medium supplemented with IL-2 was added. No additional ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator was added during the 12-day culture period (mean ± SD in 3 experiments with 7 donors).

Notes and Tips

- (Optional) Rest the post-thawed T cells overnight at 37°C in ImmunoCult™-XF before using the cells in T cell activation and expansion cultures.
- ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator supports strong T cell activation and expansion in combination with ImmunoCult™-XF, Human Recombinant IL-7 (Catalog #78053), and Human Recombinant IL-15 (Catalog #78031).

ASSESSING PURITY

ImmunoCult™ Human CD3/CD28/CD2 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) and Anti-Human CD8a Antibody, Clone RPA-T8 (Catalog #60022).