**ImmunoCult™ Human CD3/CD28 T Cell Activator**

**Human T cell activation and expansion reagent**

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Volume</th>
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<tbody>
<tr>
<td>10971</td>
<td>2 mL</td>
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<tr>
<td>10991</td>
<td>5 x 2 mL</td>
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**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 tetrameric antibody complexes that bind CD3 and CD28 cell surface ligands. Binding of the tetrameric 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 tetrameric antibody complex
- Anti-human CD28 monospecific tetrameric antibody complex

**Handling / 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. Start with purified human T cells at 1 x 10^6 cells/mL in ImmunoCult™-XF T Cell Expansion Medium (Catalog #10981) or other T cell expansion medium supplemented with cytokines (e.g. 30 - 100 IU/mL IL-2; Catalog #78036).
2. To activate human T cells, add 25 µL of ImmunoCult™ Human CD3/CD28 T Cell Activator per 1 mL of cell suspension and incubate at 37°C and 5% CO₂ for up to 3 days.
3. To expand human T cells, every 2 - 3 days adjust cell density to 1 x 10^6 cells/mL with the addition of fresh ImmunoCult™-XF T Cell Expansion Medium (or other T cell expansion medium) supplemented with cytokines and incubate cells at 37°C and 5% CO₂.
4. For longer-term expansion of human T cells, every 7 - 10 days harvest and resuspend the expanded T cells at 1 x 10^6 cells/mL in fresh culture medium and restimulate with the addition of 25 µL ImmunoCult™ Human CD3/CD28 T Cell Activator per 1 mL of cell suspension. Incubate cells at 37°C and 5% CO₂ and every 2 - 3 days adjust cell density to 1 x 10^6 cells/mL with the addition of fresh ImmunoCult™-XF T Cell Expansion Medium or other T cell expansion medium supplemented with cytokines.

**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.
FIGURE 1. Activated Morphology of 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 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 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 ± 2.4% (mean ± SD). Following 3 days of culture, the frequency of CD25-positive cells was (B) 75.4 ± 13.8% (mean ± SD) when stimulated with ImmunoCult™ Human CD3/CD28 T Cell Activator.

FIGURE 3. Robust Human T Cell Expansion with ImmunoCult™ CD3/CD28 T Cell Activator
EasySep™-isolated human T cells were expanded over 14 days with ImmunoCult™ Human CD3/CD28 T Cell Activator in ImmunoCult™-XF T Cell Expansion Medium supplemented with recombinant human IL-2. On day 0, 1 x 10^6 EasySep™-isolated human T cells were stimulated with 25 µL of ImmunoCult™ Human CD3/CD28 T Cell Activator in ImmunoCult™-XF T Cell Expansion Medium. On days 6, 9, and 11, viable cells were counted and fresh medium and cytokines were added. No additional ImmunoCult™ Human CD3/CD28 T Cell Activator was added during the 14-day culture period.