

# ImmunoCult™-XF T Cell Expansion Medium

Serum-free and xeno-free medium for the expansion of human T cells

Catalog #10981

500 mL



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## Product Description

ImmunoCult™-XF T Cell Expansion Medium is a serum-free™ and xeno-free medium optimized for the in vitro culture and expansion of human T cells isolated from peripheral blood. Recombinant cytokines, required for the optimal growth and expansion of T cells, have not been added to ImmunoCult™-XF T Cell Expansion Medium. This allows users the flexibility to prepare medium that meets their requirements.

- No need to supplement the medium with serum
- Supports robust T cell expansion with high viability after 10 - 12 days of culture
- Expanded T cells are able to produce cytokines including IFN-gamma and IL-4 upon restimulation
- Use with ImmunoCult™ Human T Cell Activators (Catalog #10970 and 10971) for bead-free activation of T cells

## Properties

**Storage:** Store at 2 - 8°C.

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

This product contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

## Directions for Use

NOTE: If precipitate is observed in the medium, centrifuge or filter using a 0.2 - 0.22 µm low protein binding polyethersulfone (PES) filter unit (e.g. Fisher 09-741-04 [0.2 µm, 250 mL]; Fisher SCGP00525 [0.22 µm, 50 mL]). This will not affect performance of the medium.

The following protocol is for the expansion of activated human T cells using ImmunoCult™-XF T Cell Expansion Medium. Depending on the experimental objectives, the protocol may need to be optimized (e.g. cell seeding density or cytokine concentration).

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. 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 µL/mL of ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator (Catalog #10970) or ImmunoCult™ Human CD3/CD28 T Cell Activator (Catalog #10971) to the cell suspension. Incubate cells at 37°C and 5% CO<sub>2</sub> for 3 days.
4. Day 3: Mix the cell suspension thoroughly and perform a viable cell count. Increase the volume of the cell suspension 8-fold (adjust the viable cell density to  $\sim 1 - 2.5 \times 10^5$  cells/mL) by adding fresh complete ImmunoCult™-XF T Cell Expansion Medium. Incubate at 37°C and 5% CO<sub>2</sub> for 2 days.

5. Day 5: Mix the cell suspension thoroughly and perform a viable cell count. Increase the volume at least 4-fold (adjust the viable cell density to  $\sim 1 - 3 \times 10^5$  cells/mL) by adding fresh complete ImmunoCult™-XF T Cell Expansion Medium. Incubate at 37°C and 5% CO<sub>2</sub> for 2 days.
6. Day 7: Mix the cell suspension thoroughly and perform a viable cell count. Increase the volume at least 4-fold (adjust the viable cell density to  $\sim 1 - 6 \times 10^5$  cells/mL) by adding fresh complete ImmunoCult™-XF T Cell Expansion Medium. Incubate at 37°C and 5% CO<sub>2</sub> for 3 days.
7. Day 10: Harvest cells if the desired cell number is achieved.  
OPTIONAL: Perform a viable cell count and maintain cell density at  $0.5 - 1 \times 10^6$  cells/mL by adding fresh complete ImmunoCult™-XF T Cell Expansion Medium. Incubate at 37°C and 5% CO<sub>2</sub> for 2 days, then harvest cells.
8. 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 µL/mL of ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator or 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

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

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