Expansion of T Cells without Serum in Chemically Defined Media

Application Note

Workflow Summary

Combine supplements of OptiPEAK T Lymphocyte media. Isolate human T lymphocytes from whole blood. Wash to remove traces of donor serum. Activate and expand T lymphocytes (7-10 days) in OptiPEAK T Lymphocyte serum free media. Harvest T lymphocytes. Perform post-expansion, flow cytometric analysis of phenotypic surface markers.

Introduction

Human T Cell expansion has become an increasingly important tool in the development of both autologous and allogeneic immunotherapies, as T cells emerge as an exciting option to treat a wide range of diseases. Major components used to expand these cells include human and bovine serum, as well as proteins derived from these substances. These components not only have the potential to introduce harmful pathogens to the already compromised patient in a clinical setting, but they also have significant limitations in the ability to ensure robust and reproducible performance in cell culture systems. Even so-called "xeno-free" media—often confused with chemically defined, animal-component-free media—contain proteins derived from human or bovine serum. OptiPEAK T Lymphocyte media (product No. 777OPT069) supports the growth and expansion of human primary T cells and contains only synthetic and recombinant components. This medium is considered blood-free and all components are chemically defined, providing robust performance without compromising growth, safety, or phenotype [1,2,3].

Materials

Growth Media Preparation

- ✔ OptiPEAK T Lymphocyte media (product No. 777OPT069) Components: Base Media, 50X Protein Supplement, 10X Media Supplement
- ✔ Gentamicin/amphotericin B (Life Technologies #R01510)

Optional:

- ✔ RPMI-medium with Glutamax
- ✔ Fetal Bovine Serum (FBS)
- ✔ Human Serum (HS)

Plate Set Up

- ✔ 12-Well flat-bottom sterile plates with lids

- 6-Well flat-bottom sterile plates with lids

Human T-Lymphocyte Isolation

- Human whole blood from 2 or more normal healthy donors
- Human T Cell Enrichment Cocktail (Stem Cell Technologies #15061)
- Density Gradient (StemCell Technologies #07801)
- Dulbecco's Phosphate Buffered Saline (dPBS)

Activation of T-Lymphocytes

- Human T-Activator CD3/CD28 beads (Life Technologies 11141D)
- Recombinant Human IL-2 (Peprotech 200-02)

Flow Cytometry Analysis

- Harvest antibodies, mouse
- CD8, PE labeled
- CD4, FITC labeled

Equipment

- Centrifuge and 50 mL conical tubes
- 5% CO2 incubator at 37°C, saturating relative humidity
- Class A2 biological safety counter
- Cell counter or hemocytometer
- Flow Cytometer

Protocol

Growth Media Preparation

1. To generate 1 liter of complete OptiPEAK T Lymphocyte, thaw one OptiPEAK T Lymphocyte Media Supplement and one OptiPEAK T Lymphocyte Protein Supplement in a 37°C water bath. Do not subject the Protein Supplement to multiple freeze-thaw cycles.
2. Once the supplements are completely thawed, gently mix the supplements by gently pipetting up and down. Do not vortex.
3. Add 100 mL from the OptiPEAK T Lymphocyte Media Supplement directly to the OptiPEAK T Lymphocyte Base Media.
4. Add 20 mL of OptiPEAK T Lymphocyte Protein Supplement directly to the OptiPEAK T Lymphocyte Base Media.
5. This medium requires the supplementation of 10ng/mL recombinant IL-2 or equivalent per desired application. This medium contains HEPES and glutamine source.
6. If desired, add gentamicin/amphotericin B (Life Technologies #R01510) at 0.1 to 0.5x final concentration. Do not add to 1x final concentration.

7. Once mixed, avoid repetitive heating and cooling of the complete medium. Instead, withdraw and prewarm only the volume needed for the specific procedure.

Plate Set up

1. In a 12-well plate, add one mL of each media into appropriate wells.
2. Place plate in incubator for warming.

Human T-Cell Isolation

1. Acquire 8-10 mL of fresh whole blood from two different healthy human donors.
2. Add T-Cell Enrichment cocktail (50 µL per 1 mL of blood) to sample and incubate at room temperature for 20 minutes [4].
3. Transfer blood sample to a 50 mL tube.
4. Rinse the blood sample tube with an equal volume of dPBS and combine rinsate with blood sample. Final volume will be approximately 20 mL.
5. In a separate tube, prepare 15 mL of gradient density medium and slowly layer PBS-diluted blood sample on top.
6. Centrifuge at 2600 RPM for 20 minutes with brake off. Allow centrifuge to come to a full rest.
7. Harvest the T-cell enriched buffy coat (the “fuzzy” cell layer at the density gradient interface) with a serological pipette and transfer to a new 50 mL conical tube.
8. Fill new tube to top with dPBS to wash enriched cells.
9. Centrifuge at 1400 RPM for 10 minutes with brake on low.
10. Discard supernatant and repeat washing steps for a total of two washes.
11. Resuspend cells in 450 µL dPBS.
12. Count cells in triplicate using a cell counter or equivalent.

T-Cell Activation

1. Calculate the volume of Human T-Activator CD3/CD28 beads necessary to achieve a ratio of 1 bead per cell [5].
2. Add volume of Human T-Activator CD3/CD28 beads to volume of cells
3. Equally distribute activated cells between each media condition across donors
4. Place the plate in the incubator.

Cell Expansion

1. Expand cells for 7 days, doubling media volume every other day.
2. Harvest cells on Day 7 and count. Stain for phenotypic markers.

Results and Discussion

Mean-fold expansion of cells in OptiPeak T Lymphocyte blood-free media was comparable to cells in 5% human serum and greater than cells in 10% fetal bovine serum (Figure 1A). Cells maintained in OptiPeak T lymphocyte blood-free media also showed CD4+ and CD8+ ratios comparable to cells grown in human and bovine serum (Figure 1B). These results show OptiPeak T Lymphocyte blood-free medium's potential as a viable and regulatory-friendly option for T cell immunotherapy manufacturing.

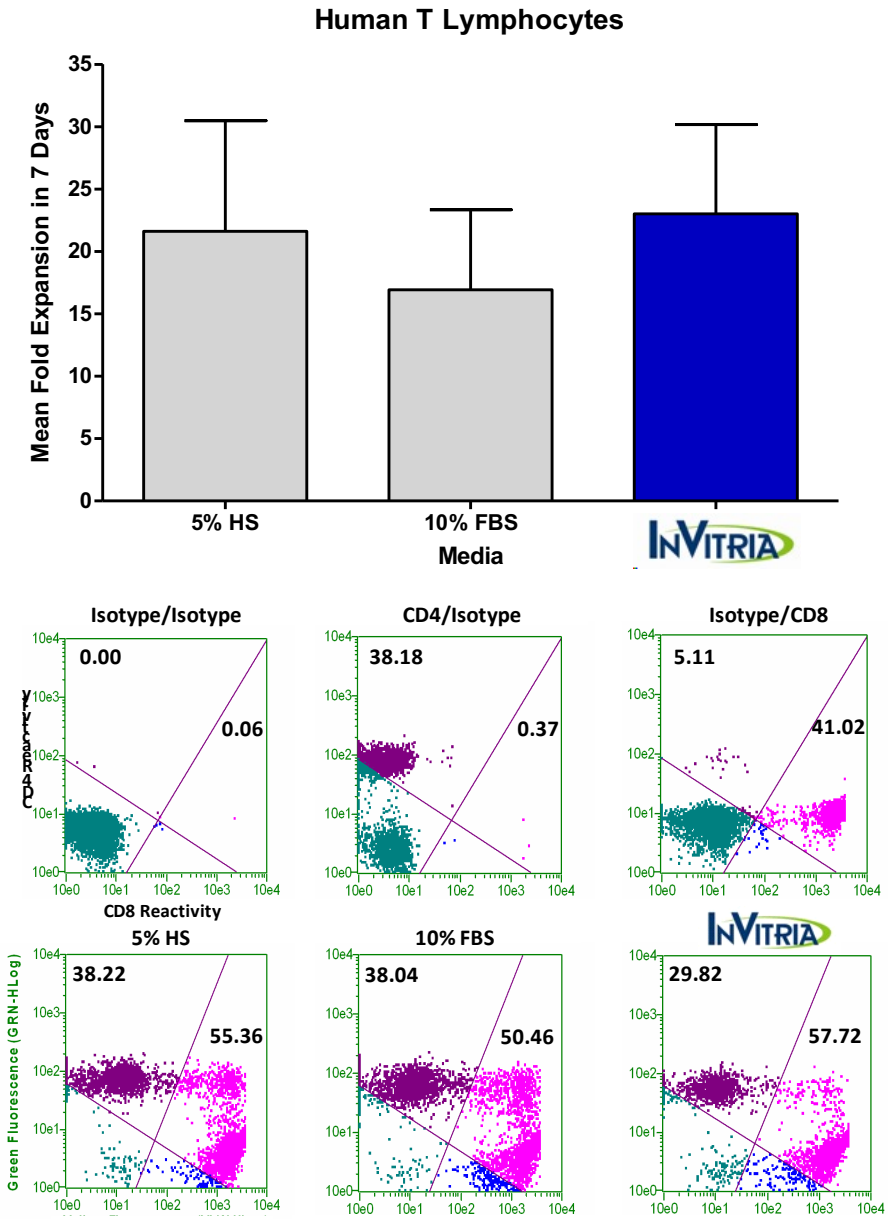

Figure 1. Performance of normal peripheral T Cells in OptiPEAK T Lymphocyte media compared to standard media supplemented with fetal bovine serum (FBS) or human serum (HS).

References

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4. “RosetteSep™ Human T Cell Enrichment Cocktail” Product Information Sheet. https://cdn.stemcell.com/media/files/pis/28570-PIS_1_3_1.pdf
5. “Dynabeads Human T-Expander CD3/CD28” Product Manual. https://assets.thermofisher.com/TFS-Assets/LSG/manuals/Dynabeads_hu_Expander_CD3CD28_man.pdf

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Annie Cunningham joined InVitria in 2018 as a Cell Culture Scientist. After graduating from Michigan State University, Annie began her career at Seattle Genetics developing potency assays for antibody-drug conjugates for cancer treatment. Annie shifted focus into immunotherapy and contributed to the development of the CAR T program at Juno Therapeutics. After moving to Colorado in 2017, Annie continued researching the human immune system as it relates to the gut microbiome at the University of Colorado. In her role at InVitria, she enjoys the new challenge of formulating completely blood-free, chemically defined cell culture media for the scalable and sustainable manufacturing of life-saving medicines.