

# OpTmizer™ CTS™ T-Cell Expansion SFM

## Description

OpTmizer™ CTS™ T-Cell Expansion SFM has been developed for the growth and expansion of human T lymphocytes. OpTmizer™ CTS™ T-Cell Expansion Medium is a complete serum-free, xenofree 1X medium consisting of OpTmizer™ CTS™ T-Cell Expansion Basal Medium with the addition of OpTmizer™ CTS™ T-Cell Expansion Supplement. Each container is a sterile filtered single use container.

Product	Catalog No.	Amount	Storage	Shelf Life*
OpTmizer™ CTS™ T-Cell Expansion SFM Contains:	A10485-01**	1 Kit		18 months
OpTmizer™ CTS™ T-Cell Expansion Basal Medium	A10221-01	1 × 1000 mL (Bottle)	2°C to 8°C; Protect from light	
OpTmizer™ CTS™ T-Cell Expansion Supplement	A10484-02	1 × 26 mL	2°C to 8°C; Protect from light	
OpTmizer™ CTS™ T-Cell Expansion SFM Contains:	A10485-03**	1 Kit		18 months
OpTmizer™ CTS™ T-Cell Expansion Basal Medium	A10221-03	1 × 1 L (Media Bag)	2°C to 8°C; Protect from light	
OpTmizer™ CTS™ T-Cell Expansion Supplement	A10484-02	1 × 26 mL	2°C to 8°C; Protect from light	

\* Shelf Life duration is determined from Date of Manufacture.

\*\* OpTmizer™ CTS™ T-Cell Expansion SFM is sold as a complete kit, components are not sold separately.

## Intended Use

For human ex-vivo tissue and cell culture processing applications.

**CAUTION:** When used as a medical device, Federal law restricts this device to sale by or on the order of a physician.

## Important Information

- **Do not freeze** OpTmizer™ T-Cell Expansion Supplement.
- Foaming may occur during shipment of the supplement, but will not impact performance of the product.
- Supports high density CD3+ T-cell cultures (e.g., >3 × 10<sup>6</sup> cells/mL) in static and (e.g., >2 × 10<sup>7</sup> cells/mL) WAVE Bioreactor™ cultures.

## Safety Information

For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Human origin materials are non-reactive (donor level) for anti-HIV 1 & 2, anti-HCV and HBsAg. Handle in accordance with established bio-safety practices.

## Prepare Media

OpTmizer™ CTS™ T-Cell Expansion Basal Medium requires supplementation with OpTmizer™ CTS™ T-Cell Expansion Supplement, and L-glutamine.

**Note:** For complete 1X medium preparation in the media bag, a needle syringe can be used to aseptically inject the supplement(s) into the media bag via the self sealing injection site.

1. For complete 1X medium, aseptically add to OpTmizer™ CTS™ T-Cell Expansion Basal Medium before use:
  - 26 mL/L of OpTmizer™ CTS™ T-Cell Expansion Supplement.
  - 10 mL/L of 200 mM L-glutamine solution for a final concentration of 2 mM.
2. Place the OpTmizer™ CTS™ T-Cell Expansion Basal Medium, OpTmizer™ CTS™ T-Cell Expansion Supplement and thawed L-Glutamine solution under a sterile laminar flow hood.
3. Remove the caps and using a sterile pipette, remove 26 mL of OpTmizer™ CTS™ T-Cell Expansion Supplement and add to 1 L of OpTmizer™ CTS™ T-Cell Expansion Basal Medium. Discard pipette.

4. Using a new sterile pipette, remove 10 mL of L-Glutamine solution and add to 1 L of OpTmizer™ CTS™ T-Cell Expansion Basal Medium. Discard pipette.
5. Replace the caps tightly and swirl gently to mix the complete OpTmizer™ CTS™ T-Cell Expansion SFM.
6. Medium can be further supplemented with cytokines and/or antibiotics if desired following the same steps 1–4 above.
7. Complete 1X OpTmizer™ CTS™ T-Cell Expansion SFM may be supplemented with cytokines such as IL-2 to support T-cell expansion. It is recommended to use 100–200 IU/mL of IL-2 for standard T cell expansion. The amount of IL-2 used may vary depending on experimental conditions.
8. If desired, antibiotics can be used. It is recommended to use Gentamicin at 10–50 µg/mL or Penicillin-Streptomycin.

**Note:** OpTmizer™ CTS™ T-Cell Expansion SFM is designed to support T-cell cultures without the addition of human serum. If required, 2% heat-inactivated human serum may be added to the medium to enhance viability and expansion. The use of serum, and the amount required, should be determined empirically depending on the specific T-cell culture application.

Complete OpTmizer™ CTS™ T-Cell Expansion SFM (basal medium with supplement, and L-glutamine) is stable for 4 weeks when stored in the dark at 2°C to 8°C.

## Culture Conditions

**Media:** Complete OpTmizer™ CTS™ T-Cell Expansion SFM

**Cells:** Peripheral Blood Mononuclear Cells (PBMC)

**Culture Type:** Suspension

**Culture Vessels:** T-Flasks or WAVE Cellbag® Bioreactor

**Temperature Range:** 36°C to 38°C

**Incubator Atmosphere:** Humidified atmosphere of 5% CO<sub>2</sub> in air. Ensure proper gas exchange and minimize exposure of cultures to light.

## Culture Procedure

**Note:** The procedure below serves as a general guideline for all static T-cell cultures, regardless of vessel. For high-density culture in bioreactors, such as WAVE Cellbag®, optimal procedures should be determined empirically by the investigator.

1. Prepare fresh peripheral blood mononuclear cells (PBMCs) or rapidly thaw (<1 minute) frozen vials of PBMCs cells in a 37°C water bath according to standard PBMC thawing protocols.
2. Wash cells with Dulbecco's Phosphate Buffered Saline (DPBS) without calcium and magnesium, with 5% heat-inactivated FBS or heat-inactivated human pooled Type AB serum according to the applications, if desired or required.
3. Determine total viable cell density and cell viability using Countess® Automated Cell Counter. Centrifuge cells at 200 × g for 5–10 minutes and remove wash buffer.
4. Resuspend PBMCs at 0.5–1 × 10<sup>6</sup> cells/mL in 1X complete OpTmizer™ CTS™ T-Cell Expansion SFM, supplemented with cytokines if used at culture initiation. Transfer the desired number of cells to the desired tissue culture vessel.

**Note:** A variety of protocols may be used for activating T-cells for subsequent expansion, including adding stimulatory antibodies or antigen presenting cells. Similarly, for either small or the large scale T-cell expansion, cells can be isolated, activated and expanded with Dynabeads® CD3/CD28 CTS™ according to instructions in the product insert.

5. Incubate the culture vessel at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> in air.
6. Feed and maintain cells at desired concentrations while cells are in log phase growth. To maintain log phase growth, it may be preferable to split cells to achieve a density of 0.5–1 × 10<sup>6</sup> T-cells/mL whenever cell density gets above 1 × 10<sup>6</sup> cells/mL (e.g. 2 × 10<sup>6</sup> cells/mL would be split 1:4 to continue culture at 0.5 × 10<sup>6</sup> cells/mL).

**Note:** For optimal gas exchange in static plate cultures it is recommended that medium depth not exceed 1–1.2 cm.

## Related Products

Product	Catalog No.
Dulbecco's Phosphate Buffered Saline CTS™, without calcium and magnesium	A12856
L-Glutamine, 200 mM (100X), liquid	25030
GlutaMAX™-I CTS™, 200 mL (100X), liquid	A12860
IL-2 (Interleukin 2) CTS™ Recombinant Human	CTP0021
IL-7 (Interleukin 7) CTS™ Recombinant Human	CTP0071
AB-Human Serum	34005
Certified FBS, Heat Inactivated, US	10082
Gentamicin Reagent Solution (50 mg/mL). liquid	15750
Penicillin-Streptomycin 100X Solution	15070
Countess® Automated Cell Counter	C10227
Dynabeads® CD3/CD28 CTS™	402-03D
DynaMag™ CTS™	121-02
Dynabeads® Human Treg Expander	111-29D

For additional information related to T-cell expansion using Life Technologies products refer to our website:

[www.lifetechnologies.com/gibcocts](http://www.lifetechnologies.com/gibcocts).

For information using Dynabeads® CD3/CD28 CTS™ (402-03D) refer to our website:

[www.lifetechnologies.com/celltherapyresearchsupport](http://www.lifetechnologies.com/celltherapyresearchsupport)

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For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit [www.lifetechnologies.com/celltherapyresearchsupport](http://www.lifetechnologies.com/celltherapyresearchsupport). For further assistance, email [techsupport@lifetech.com](mailto:techsupport@lifetech.com)

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