


CTS™ OpTmizer™ Pro Serum Free Medium, No Phenol Red

Catalog Numbers A4966101, A4966103

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 **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](https://www.thermofisher.com/support).

Product description

The Gibco™ CTS™ OpTmizer™ Pro Serum Free Medium, No Phenol Red is a novel medium developed for the growth and expansion of human T lymphocytes. CTS™ OpTmizer™ Pro Serum Free Medium, No Phenol Red media helps improve central memory phenotype and cell growth by shifting the cellular metabolism. CTS™ OpTmizer™ Pro Serum Free Medium, No Phenol Red is a complete serum-free, xeno-free medium consisting of CTS™ OpTmizer™ Pro Basal Medium (No Phenol Red) with the addition of CTS™ OpTmizer™ T-Cell Expansion Supplement. Each container is a sterile filtered single-use container.

Contents and storage

CTS™ OpTmizer™ Pro Serum Free Medium, No Phenol Red is sold as a complete kit. The components are not sold separately.

Contents	Amount	Storage	Shelf life ^[1]
CTS™ OpTmizer™ Pro Serum Free Medium, No Phenol Red, Cat. No. A4966101			
CTS™ OpTmizer™ Pro Basal Medium	1000 mL (Bottle)	2°C to 8°C. Protect from light.	12 months
CTS™ OpTmizer™ T-Cell Expansion Supplement	26 mL		
CTS™ OpTmizer™ Pro Serum Free Medium, No Phenol Red, Cat. No. A4966103			
CTS™ OpTmizer™ Pro Basal Medium	1 L (Media Bag)	2°C to 8°C. Protect from light.	12 months
CTS™ OpTmizer™ T-Cell Expansion Supplement	26 mL		

^[1] Shelf-Life duration is determined from Date of Manufacture.

Safety information

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.

Culture conditions

Media: Complete CTS™ OpTmizer™ Pro Serum Free Medium, No Phenol Red

Cells: Peripheral Blood Mononuclear Cells (PBMC) or T cells (any type)

Culture type: Suspension

Culture vessels: T-Flasks or Xuri™ Cellbag™ Bioreactor

Temperature range: 36°C to 38°C

Incubator atmosphere: Humidified atmosphere of 5% CO₂ in air. Ensure proper gas exchange and minimize exposure of cultures to light.

Procedural guidelines

- Do not freeze CTS™ OpTmizer™ T-Cell Expansion Supplement.
- Foaming may occur during shipment of the supplement, but will not impact performance of the product.
- Product supports high density CD3⁺ T-cell cultures (e.g., >3 × 10⁶ cells/mL) in static cultures and (e.g., >2 × 10⁷ cells/mL) WAVE Cellbag™ cultures.

Prepare media

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

Note: To prepare complete 1X medium in the media bag, use a needle syringe to aseptically inject the supplement(s) into the media bag via the self-sealing injection site.

1. Place the CTS™ OpTmizer™ Pro Basal Medium, CTS™ OpTmizer™ T-Cell Expansion Supplement, and thawed L-glutamine (200 mM) under a sterile laminar flow hood.
2. Add 26 mL CTS™ OpTmizer™ T-Cell Expansion Supplement to 1 L CTS™ OpTmizer™ Pro Basal Medium.
Discard pipette.
3. Use a new sterile pipette to remove 10 mL of L-glutamine (200 mM) and add to 1 L of CTS™ OpTmizer™ Pro Basal Medium.
Discard pipette.
4. Replace the caps tightly and swirl gently to mix the complete CTS™ OpTmizer™ Pro Serum Free Medium.
5. Medium can be further supplemented with cytokines and/ or antibiotics if desired following steps 1–3.
6. Complete 1X CTS™ OpTmizer™ Pro Serum Free Medium 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.
Once the complete CTS™ OpTmizer™ Pro Serum Free Medium (CTS™ OpTmizer™ Pro Basal Medium with CTS™ OpTmizer™ T-Cell Expansion Supplement, and L-glutamine) is prepared in accordance with our instructions, it must be stored in the dark at 2°C to 8°C and used within four weeks of supplementing to be covered by our warranty.

Culture T-cells

General guideline for all static T-cell cultures, regardless of vessel. For high-density culture in bioreactors, such as WAVE Cellbag™ Bioreactor, optimal procedures should be determined empirically by the investigator.

1. Prepare fresh peripheral blood mononuclear cells (PBMCs)/ any kind of T-cells or rapidly thaw (<1 minute) frozen vials of cells in a 37°C water bath according to standard thawing protocols.
2. Wash cells with DPBS, no calcium, no 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.
4. Centrifuge cells at 200 × g for 5–10 minutes and remove wash buffer.
5. Resuspend PBMCs at 0.5–1 × 10⁶ cells/mL in 1X complete CTS™ OpTmizer™ Pro Serum Free Medium, supplemented with cytokines if used at culture initiation.

6. Transfer the required number of cells to the appropriate tissue culture vessel.
Note: A variety of protocols may be used to activate 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 CTS™ Dynabeads™ CD3/CD28 according to instructions in the product insert.
7. Incubate the culture vessel at 37°C in a humidified atmosphere of 5% CO₂ in air.
8. 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 × 10⁶ cells/mL whenever cell density gets above 1 × 10⁶ cells/mL (e.g. 2 × 10⁶ cells/mL would be split 1:4 to continue culture at 0.5 × 10⁶ 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

Unless otherwise indicated, all materials are available through thermofisher.com.

Item	Source
CTS™ DPBS without calcium chloride, without magnesium chloride	A12856
L-Glutamine	25030
CTS™ GlutaMAX™-I Supplement	A1286001
Human IL-2 Recombinant Protein	PHC0021
Human IL-7 Recombinant Protein	PHC0075
CTS™ Dynabeads™ CD3/CD28	40203D
CTS™ DynaMag™ Magnet	12102
Dynabeads™ Human T-Expander CD3/CD28	11141D

Limited product warranty

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