CTS™ AIM V™ Medium

Serum-free medium without Gentamicin sulfate, Phenol red, and Streptomycin sulfate

Catalog Number A4672701

Pub. No. MAN0018999 Rev. 1.0

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**.

Product description

Gibco™ CTS™ AIM V™ Medium is the first commercially available defined serum-free medium for proliferation and/or manipulation of T-cells and dendritic cells, manufactured in compliance with cGMP (21 CFR part 820). CTS™ AIM V™ Medium contains L-glutamine at 0.3358 mg/mL. CTS™ AIM V™ Medium does not contain gentamicin sulfate, phenol red, nor streptomycin sulfate. Each container is a sterile filtered single-use container.

Contents and storage

Contents	Cat. No.	Amount	Storage	Shelf life[1]
CTS™ AIM V™ Medium	A4672701	2 L	2°C to 8°C; Protect from light	12 months

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

Safety information

Human origin raw 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: CTS™ AIM V™ Medium

Cells: Human peripheral blood mononuclear cells (PBMCs)

Culture type: Static suspension

Culture vessels: T-Flasks or cell culture bag

Temperature range: 36°C to 38°C

Incubator atmosphere: Humidified atmosphere of 4–6% CO_2 in air. Ensure proper gas exchange and minimize exposure of cultures to light.

Procedural guidelines

- CTS™ AIM V™ Medium comes supplemented with L-glutamine.
- Additional supplementation with cytokines or growth factors may be required per specific investigator's procedures and should be aseptically added immediately prior to use.
- The following protocol serves as a general guideline for static T cell and dendritic cell culture, regardless of vessel.
- Feed and maintain cells at desired concentrations while cells are in log phase growth. Dilute cells to a viable cell density of 5×10^5 cells/mL whenever the viable cell density reaches $\geq 1 \times 10^6$ cells/mL.
- For optimal gas exchange in static plate cultures, it is recommended that medium depth not exceed 1–1.2 cm.
- For high-density culture in bioreactors, optimal procedures should be determined empirically by the investigator.

T-cell culture

1. Prepare fresh peripheral blood mononuclear cells (PBMCs) or rapidly thaw (<1 minute) a vial of frozen PBMCs in a 37°C water bath according to standard PBMC thawing protocols.

2. Wash cells with CTS™ DPBS without calcium chloride, without magnesium chloride, supplemented with 2–5% heat-inactivated human pooled Type AB serum or 5–10% CTS™ Immune Cell SR.

   The optimal concentration should be determined based on your application.

3. Determine viable cell density using a Countess™ II Automated Cell Counter.

4. Centrifuge cells at 200 × g for 5–10 minutes and aspirate wash buffer supernatant.

5. Resuspend PBMC pellet at approximately 0.5×10^6–1×10^6 cells/mL in medium supplemented with cytokines (e.g., IL-2), if used at culture initiation.

6. 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 large scale T-cell expansion, cells can be isolated, activated and expanded using CTS™ Dynabeads™ CD3/CD28 according to instructions in the product insert.

Prepare monocyte derived dendritic cell culture

1. Prepare fresh PBMCs and seed into a culture flask with 25 mL RPMI 1640 or CTS™AIM-V™ Medium (Therapeutic Grade) at cell density of $1–2 \times 10^5$ cells/cm^2.

2. Incubate for 2–3 hours at 37°C in a humidified atmosphere of 5% CO_2 in air.

3. Aspirate and discard medium containing non-adherent cells.

4. Wash the adherent cells (mainly CD14+ monocytes) three times with CTS™ DPBS without calcium chloride, without magnesium chloride.

5. Add medium supplemented with 50–100 ng/mL recombinant human IL-4 and 50 ng/mL recombinant human GM-CSF.

6. Incubate cells at 37°C in a humidified atmosphere of 5% CO_2 in air for 5 days.

7. On day 3, transfer spent media to a sterile conical tube and centrifuge at 200 × g for 5–10 minutes to collect all non-adherent or loosely adherent cells.

8. Aspirate supernatant and gently resuspend cell pellet in an equal volume of fresh pre-warmed medium containing IL-4 and GM-CSF.

9. Transfer cell suspension to the original culture flask containing adherent cells.

   After 6 days, the loosely adherent or non-adherent cells should display typical dendritic cell morphology and surface markers (e.g., CD1a, CD80, CD86, and HLA-DR).

10. Induce maturation of dendritic cells by the addition of either 1 µg/mL lipopolysaccharide (LPS) or 50 µL/mL CTS™ TNF-α to the medium.

    Note: As an alternative to plastic adherence, monocytes can be isolated by magnetic separation.

Related products

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

Item	Source
CTS™ DPBS without calcium chloride, without magnesium chloride	A12856
CTS™ IL-2 Recombinant Human	CTP0021
CTS™ IL-4 Recombinant Human	CTP0041
CTS™ IL-7 Recombinant Human	CTP0071
CTS™ GM-CSF Recombinant Human	CTP2011
CTS™ TNF Recombinant Human	CTP3011
CTS™ Immune Cell SR	A2596101
CTS™ Dynabeads™ CD3/CD28	40203D
CTS™ DynaMag™ Magnet	12102
Dynabeads™ Human Treg Expander	11129D
RPMI 1640 Medium, GlutaMAX™ Supplement, HEPES	72400
CTS™ GlutaMAX™-I Supplement	A1286001
L-Glutamine (200 mM) (100X)	25030
Trypan Blue Solution, 0.4%	15250
Countess™ II Automated Cell Counter	AMQAX1000

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

The information in this guide is subject to change without notice.