


# CTS™ AIM V™ SFM

Catalog Numbers A3021001, A3021002

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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

CTS™ AIM V™ SFM 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. It contains L-glutamine, 50 µg/mL streptomycin sulfate, and 10 µg/mL gentamicin sulfate. Each container is a sterile filtered single use container.

## Contents and storage

Contents	Cat. No.	Amount	Storage	Shelf life <sup>[1]</sup>
CTS™ AIM V™ SFM	A3021002	1000 mL	2°C to 8°C; Protect from light	14 months
	A3021001	10 L		

<sup>[1]</sup> 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:** CTS™ AIM V™ SFM

**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<sub>2</sub> in air. Ensure proper gas exchange and minimize exposure of cultures to light.

## Procedural guidelines

- CTS™ AIM V™ SFM comes supplemented with L-glutamine, streptomycin sulfate, and gentamicin sulfate.
- 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  $5 \times 10^5$  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

- 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.
- Wash cells with CTS™ DPBS without calcium chloride, without magnesium chloride, supplemented with 2–5% heat-inactivated human pooled Type AB serum according to the application, if desired or required.
- Determine viable cell density using a Countess™ Automated Cell Counter.
- Centrifuge cells at  $200 \times g$  for 5–10 minutes and aspirate wash buffer supernatant.
- Resuspend PBMC pellet at approximately  $0.5\text{--}1 \times 10^6$  cells/mL in medium supplemented with cytokines (e.g., IL-2), 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 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

- Prepare fresh PBMCs and seed into a culture flask with 25 mL RPMI 1640 or CTS™ AIM V™ SFM at cell density of  $1-2 \times 10^5$  cells/cm<sup>2</sup>.
- Incubate for 2–3 hours at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> in air.
- Aspirate and discard medium containing non-adherent cells.
- Wash the adherent cells (mainly CD14<sup>+</sup> monocytes) three times with CTS™ DPBS without calcium chloride, without magnesium chloride.
- Add medium supplemented with 50–100 ng/mL recombinant human IL-4 and 50 ng/mL recombinant human GM-CSF. Incubate cells at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> in air for 5 days.
- Incubate cells at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> in air for 5 days.
- On day 3, transfer spent media to a sterile conical tube and centrifuge at  $200 \times g$  for 5–10 minutes to collect all non-adherent or loosely adherent cells.
- Aspirate supernatant and gently resuspend cell pellet in and equal volume of fresh prewarmed medium containing IL-4 and GM-CSF.
- 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).

- 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

Item	Source
CTS™ DPBS without calcium chloride, without magnesium chloride	A12856
IL-2 CTS™ Recombinant Human	CTP0021
IL-4 CTS™ Recombinant Human	CTP0041
IL-7 CTS™ Recombinant Human	CTP0071
GM-CSF CTS™ Recombinant Human	CTP2011
TNF CTS™ Recombinant Human	CTP3011
CTS™ Dynabeads™ CD3/CD28	40203D
CTS™ Immune Cell SR	A3021101
CTS™ DynaMag™ Magnet	12102
Dynabeads™ Human Treg Expander	11129D
RPMI 1640 Medium, GlutaMAX™ Supplement, HEPES	72400
CTS™ GlutaMAX™-I Supplement	A12860
L-Glutamine (200 mM)	A29168
Trypan Blue Solution, 0.4%	15250
Countess™ II Automated Cell Counter	AMQAX1000

## Limited product warranty

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