

# AIM-V<sup>®</sup> Medium CTS™

## Therapeutic Grade serum free cell expansion medium

GIBCO<sup>®</sup> AIM-V Medium CTS<sup>™</sup> (Therapeutic Grade) is the first commercially available defined, serum-free formulation for proliferation and/or manipulation of T-cells and dendritic cells and manufactured in compliance with cGMP. AIM-V Medium CTS<sup>™</sup> is an FDA 510(k) cleared device which is intended for human ex-vivo tissue & cell culture processing applications.

Description	Cat. No.	Size
AIM-V <sup>®</sup> Medium CTS <sup>™</sup> , Liquid	0870112DK	1000mL
AIM-V <sup>®</sup> Medium CTS <sup>™</sup> , Liquid	0870112BK	10L (Bag)

## **Intended Use**

For human ex-vivo tissue & 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.

## Storage

Store medium at 2 to 8°C. Protect from light.

## Shelf Life

14 months

## **Culture Procedure:**

The procedure below serves as a general guideline for static Tcell and dendritic cell culture, regardless of vessel. For highdensity culture in bioreactors, optimal procedures should be determined empirically by the investigator.

#### **T Cells Culture:**

- 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.
- Wash cells with DPBS CTS<sup>™</sup> without calcium and magnesium (Cat. No A12856), with 2-5% heat-inactivated human pooled Type AB serum according to the applications, if desired or required.
- 3. Count cells using either electronic (i.e. Coulter Counter, Vi-Cell) or manual (i.e. hemocytometer) methods.
- 4. Centrifuge cells and remove wash buffer.
- 5. Resuspend PBMC at roughly 0.5-1x10<sup>6</sup> CD3+ T 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. 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® ClinExVivo<sup>™</sup> CD3/CD28 or Dynabeads® CD3/CD28 CTS<sup>TM</sup> (Cat. No. 402-03D) according to instructions in the product insert.

6. Incubate the culture vessel at  $37^{\circ}$ C in a humidified atmosphere with 5% CO<sub>2</sub>. 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 0.5-1x10<sup>6</sup> cells/mL whenever cell density gets above 1x10<sup>6</sup> cells/mL (e.g. 2x10<sup>6</sup> cells/mL, split 1:4 to continue culture at 0.5x10<sup>6</sup> cells/mL). For optimal gas exchange in static plate cultures it is recommended that medium depth not exceed 1 to 1.2cm.

## Monocyte Derived Dendritic Cell Culture:

- 1. Prepare fresh peripheral blood mononuclear cells (PBMCs).
- Plate PBMC in culture flask with 25 mL RPMI 1640 (Cat. No 72400) or AIM-V<sup>®</sup> Medium CTS<sup>™</sup> (Therapeutic Grade).
- 3. Incubate for 2 to 3 hours at 36 to  $38^{\circ}$ C in a humidified atmosphere of 5% CO<sub>2</sub> in air.
- 4. Discard medium containing non-adherent cells.
- Wash the adherent cells (mainly CD14+ monocytes) three times with DPBS without calcium and magnesium (Cat. No A12856).
- Add medium containing 50 to 100 ng/mL recombinant human IL-4 (Cat. No. CTP0043 1mg or Cat. No. CTP0041 100ug) and 50 ng/mL recombinant human GM-CSF (Cat. No. CTP2011 100ug or Cat. No. CTP2013 1mg). Cell density should be between 1 to 3x10<sup>5</sup> cells/mL.
- Incubate cells at 36 to 38°C in a humidified atmosphere of 5% CO<sub>2</sub> in air for 5 days. It is recommended to replace medium once after 3 days with fresh medium containing IL-4 and GM-CSF. Save all non-adherent or loosely adherent cells by centrifuging the removed culture medium 10 minutes at 200xg and adding the pellet to the fresh culture medium.
- After 6 days, the loosely adherent or non-adherent cells should display typical dendritic cell morphology and surface markers (CD1a, CD80, CD86, and HLA-DR).
- 9. The maturation of dendritic cells is induced by the addition of either 1  $\mu$ g/mL LPS or 50 $\mu$ l/mL TNF- $\alpha$  (cat. No. CTP3013 1mg or Cat. No. CTP3011 100ug) to the medium.
- **Note:** Alternatively to plastic adherence, monocytes can also be isolated by magnetic separation.

#### **Related Products**

Dulbecco's Phosphate Buffered Saline CTS™ (DPBS) without calcium, magnesium (1X), liquid (A12856)

L-Glutamine-200mM (100X), liquid (25030)

Dynabeads<sup>®</sup> ClinExVivo<sup>™</sup> CD3/CD28 or Dynabeads<sup>®</sup> CD3/CD28 CTS<sup>™</sup>(402-03D)

DynaMag<sup>™</sup> CTS<sup>™</sup> (121-02)

IL-2 CTS<sup>™</sup> REC HU (CTP0021 100ug or CTP0023 1mg)

IL-7 CTS<sup>™</sup> REC HU (CTP0071 100ug or CTP0073 1mg)

IL-4 CTS<sup>™</sup> REC HU (CTP0041 100ug or CTP0043 1mg)

GM-CSF CTS<sup>™</sup> REC HU (CTP2011 100ug or CTP2013 1mg)

TNF-α CTS<sup>™</sup> (CTP3011 100ug or CTP3013 1mg)

#### **Technical Support**

For additional product and technical information, such as Material Safety Data Sheets (MSDS), Certificate of Analysis, etc, please visit our website at http://www.invitrogen.com/celltherapysupport/. For further assistance, please email our Technical Support team at celltherapysupport@lifetech.com

The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners

#### References

- Rebecca J et al., (2010) Natural exposure to cutaneous anthrax gives long lasting T cell immunity encompassing infection-specific Epitopes. J. Immunol., 184: 3814 – 3821
- Fabricius D et al., (2010) Prostaglandin E2 inhibits IFN-α secretion and Th1 costimulation by human plasmacytoid dendritic cells via E-prostanoid 2 and Eprostanoid 4 receptor engagement. J. Immunol., 184: 677 – 684
- Nesbit L et al., (2010) Polyfunctional T Lymphocytes Are in the Peripheral Blood of Donors Naturally Immune to Coccidioidomycosis and Are Not Induced by Dendritic Cells. Infect. Immun., 78: 309 - 315
- Jahrsdorfer B et al., (2010) Granzyme B produced by human plasmacytoid dendritic cells suppresses T-cell expansion. *Blood*, 115: 1156 – 1165
- Csillag A et al., (2010) Pollen-Induced Oxidative Stress Influences Both Innate and Adaptive Immune Responses via Altering Dendritic Cell Functions. J. Immunol., 184: 2377 – 2385
- Cornberg M et al., (2010) CD8 T Cell Cross-Reactivity Networks Mediate Heterologous Immunity in Human EBV and Murine Vaccinia Virus Infections. J. Immunol., 184: 2825 - 2838.
- Bellone S et al., (2009) Human Papillomavirus Type 16 (HPV-16) Virus-Like Particle L1-Specific CD8<sup>+</sup> Cytotoxic T Lymphocytes (CTLs) Are Equally Effective as E7-Specific CD8<sup>+</sup> CTLs in Killing Autologous HPV-16-Positive Tumor Cells in Cervical Cancer Patients: Implications for L1 Dendritic Cell-Based Therapeutic Vaccines. J. Virol., 83: 6779 - 6789
- Sato K et al., (2009) Impact of culture medium on the expansion of T cells for immunotherapy. Cytotherapy 11: 4-11
- Liu ZW et al., (2009) A CD26-Controlled Cell Surface Cascade for Regulation of T Cell Motility and Chemokine Signals. J. Immunol., 183: 3616 - 3624.
- Megyeri M et al., (2009) Complement Protease MASP-1 Activates Human Endothelial Cells: PAR4 Activation Is a Link between Complement and Endothelial Function. J. Immunol., 183: 3409 - 3416.
- Manfred L et al., (2005) Functional characterization of monocyte-derived dendritic cells generated under serum free culture conditions. *Immunology letters* 99: 209-216
- 12. Nagorsen D et al., (2003) Biased epitope selection by recombinant vaccinia-virus (rVV)-infected mature or immature dendritic cells. *Gene Therapy* 10: 1754-1765
- Lotem M et al., (2006) Presentation of tumor antigens by dendritic cells genetically modified with viral and nonviral vectors. J immunotherapy 29: 616-627
- Dietze B et al. (2008) An improved method to generate equine dendritic cells from peripheral blood mononuclear cells: divergent maturation programs by IL-4 and LPS. *Immunobiology* 213:751–758.
- Meehan KR et al. (2008) Development of a clinical model for *ex vivo* expansion of multiple populations of effector cells for adoptive cellular therapy. *Cytotherapy* 10: 30–37.
- Ye Z et al. (2006) Human dendritic cells engineered to express alpha tumor necrosis factor maintain cellular maturation and T-cell stimulation capacity. *Cancer Biother Radiopharm* 21:613–622.

- Choi BH et al. (2006) Optimization of the concentration of autologous serum for generation of leukemic dendritic cells from acute myeloid leukemic cells for clinical immunotherapy. J Clin Apher 21:233–240.
- Imataki O et al. (2006) Efficient ex vivo expansion of alpha24+ NKT cells derived from G-CSF-mobilized blood cells. J Immunother 29:320–327.
- Peng JC et al. (2005) Generation and maturation of dendritic cells for clinical application under serum-free conditions. J Immunother 28:599–609.
- Trickett AE et al. (2002) Ex vivo expansion of functional T lymphocytes from HIV infected individuals. J Immunol Methods 262:71–83.
- Carlens S et al. (2000) Ex vivo T lymphocyte expansion for retroviral transduction: influence of serum-free media on variations in cell expansion rates and lymphocyte subset distribution. Exp Hematol 28:1137–1146.
- Kambe N et al. (2000) An improved procedure for the development of human mast cells from dispersed fetal liver cells in serum-free culture medium. J Immunol Methods 240:101–110.
- Gerin PA et al. (1999) Production of retroviral vectors for gene therapy with the human packaging cell line FLYRD18. *Biotechnol Prog* 15:941–948.
- Slunt JB et al. (1997) Human T-cell responses to *Trichophyton tonsurans:* inhibition using the serum free medium Aim-V. *Clin Exp Allergy* 27:1184–1192.
- Kreuzfelder E (1996) Assessment of peripheral blood mononuclear cell proliferation by [2-3H]adenine uptake in the woodchuck model. *Clin Immunol Immunopathol* 78:223–227.
- Causey AL (1994) A serum-free medium for human primary T lymphocyte culture. J Immunol Methods 175:115–121.
- Freedman RS et al. (1994) Large-scale expansion in interleukin-2 of tumorinfiltrating lymphocytes from patients with ovarian carcinoma for adoptive immunotherapy. *J Immunol Methods* 167:145–160.
- Nomura K et al. (1993) [Study of adoptive immunotherapy for metastatic renal cell carcinoma with lymphokine-activated killer (LAK) cells and interleukin-2. II. Clinical evaluation.] Nippon Hinyokika Gakkai Zasshi 84:831–840. Japanese.
- Kaldjian EP et al. (1992) Enhancement of lymphocyte proliferation assays by use of serum-free medium. *J Immunol Methods* 147:189–195.
- Hayakawa K et al. (1991) Study of tumor-infiltrating lymphocytes for adoptive therapy of renal cell carcinoma (RCC) and metastatic melanoma: sequential proliferation of cytotoxic natural killer and noncytotoxic T cells in RCC. J Immunother10:313–325.
- McVicar DW et al. (1991) A comparison of serum-free media for the support of *in* vitro mitogen-induced blastogenic expansion of cytolytic lymphocytes. Cytotechnology 6:105–113.
- Burg S et al. (1991) [Effect of different media on long-term cultivation of human synovial macrophages.] Z Rheumatol 50:142–150. German.
- Helinski EH et al. (1988) Long-term cultivation of functional human macrophages in Teflon dishes with serum-free media. J Leukoc Biol 44:111–121.
- Robyn S et al. (2007) RA8, A human anti-CD25 antibody against human Treg cells. *Hybridoma* 26:119–130.
- Chena X et al. (2006) Induction of primary anti-HIV CD4 and CD8 T cell responses by dendritic cells transduced with self-inactivating lentiviral vectors. *Cell Immunol* 243:10–18.
- Grant R et al. (2008) CCL2 increases X4-tropic HIV-1 entry into resting CD4+ T cells. J Biol Chem 283:30745–30753.
- Hagihara M et al. (2003) Increased frequency of CD3/8/56-positive umbilical cord blood T lymphocytes after allo-priming *in vitro*. Ann Hematol 82:166–170.
- Wang Z et al. (2006) Application of serum-free culture medium for preparation of A-NK cells. Cell Mol Immunol 3:391–395.
- Morecki S et al. (1991) Retrovirus-mediated gene transfer into CD4+ and CD8+ human T cell subsets derived from tumor-infiltrating lymphocytes and peripheral blood mononuclear cells. *Cancer Immunol Immunother* 32:342–352.
- Johansen P et al. (2003) CD4 T cells guarantee optimal competitive fitness of CD8 memory T cells. Eur J Immunol 34:91–97.

June 2010

Form No. 5047