Miltenyi Biotec

PepTivator® CMV pp65 – HT

human

Order no. 130-097-727

Contents

1. Description

This product is for research use only.

Components	**1×96-well plate, consisting of 12 strips of 8 wells** Pool of lyophilized peptides, consisting mainly of 15-mer sequences with 11 amino acids (aa) overlap, covering the complete sequence of the pp65 protein of human cytomegalovirus (CMV) strain AD169 (Swiss-Prot Acc. no. P06725).
Capacity	96 wells containing 0.06 nmol (0.1 µg) per peptide per well. One well is for stimulation of up to 10^6 total cells.
Product format	Lyophilized peptides containing stabilizer.
Purity	Each peptide >80% (HPLC), low endotoxin.
Storage	Store lyophilized product at –20 °C. Protect from humidity. The expiration date is indicated on the product label.

This product contains no preservatives; always handle under aseptic conditions.

1.1 Background information

The PepTivator – HT Peptide Pools are designed for the simple stimulation of antigen-specific T cells. The convenient 96-well format, composed of 12 individually removable strips of 8 wells each allows for easy and flexible experimental set-up. Thus different antigens can be combined in one plate.

CMV is a member of the herpesvirus group and belongs to the subfamily of beta-herpesviruses. Between 50–85% of human adults are infected with CMV. Once infected, the virus persists in the organism. The infection is asymptomatic in healthy individuals, but in immunocompromised patients CMV can cause severe diseases.

CMV pp65 (65 kDa lower matrix phosphoprotein)[1], also known as glycoprotein 64 or UL83, is a virion tegument protein and the main component of the enveloped subviral particle. CMV pp65 is an immunodominant target of $CD4^+$ as well as $CD8^+$ T cell responses to CMV.[2] CMV pp65-specific T cells predominantly produce inflammatory cytokines like IFN-γ, IL-2, and TNF-α.

The PepTivator Peptide Pools have been specially developed for efficient *in vitro* stimulation of antigen-specific $CD4^+$ and $CD8^+$ T cells, as peptides of 15 amino acid length with 11 amino acid overlap represent the optimized solution for stimulating both $CD4^+$ and $CD8^+$ T cells in various applications. Stimulation of T cells with PepTivator Peptide Pools causes the secretion of effector cytokines and upregulation of activation markers, which then allows the detection or isolation of antigen-specific T cells. Quantitative, phenotypical, or functional analysis of antigen-specific T cell immunity can provide important information on the natural course of immune responses in healthy or immunocompromised individuals.

1.2 Applications

- Detection and analysis of antigen-specific $CD4^+$ and $CD8^+$ effector/memory T cells, for example, in peripheral blood mononuclear cells (PBMCs), by MACS® Cytokine Secretion Assays, intracellular cytokine staining, or other technologies.
- Immunomonitoring of various antigen specificities in one plate.
- Generation of antigen-specific $CD4^+$ and $CD8^+$ effector/memory T cells from naive T cell populations for research on immunotherapy and vaccination.
- Pulsing of antigen-presenting cells for research on dendritic cell vaccination.

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

140-003-658.02

2. Recommendations for *in vitro* restimulation of antigen-specific T cells with PepTivator Peptide Pools

2.1 Cell preparation

For induction of cytokine secretion by antigen-specific T cells, best results are achieved by stimulation of fresh PBMCs, whole blood, or other leukocyte-containing single-cell preparations from tissues or cell lines. Alternatively, frozen cell preparations can be used.

▲ **Note:** Remove platelets after density gradient separation. Resuspend cell pellet, fill tube with buffer, and mix. Centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully remove supernatant.

▲ **Note:** PBMCs may be stored overnight. The cells should be resuspended and incubated in culture medium as described in 2.4, steps 1–3, but without addition of antigen. The antigen is then added to the culture on the next day.

For details about cell preparation refer to the protocols section at www.miltenyibiotec.com/protocols.

2.2 Reagent requirements

- Culture medium, e.g., TexMACS™ Medium (# 130-097-196) or RPMI 1640 containing 5% human serum, e.g., autologous or AB serum.

  ▲ **Note:** Do not use bovine serum albumin (BSA) or fetal bovine serum (FBS) because of non-specific stimulation.

- Control plate (8×12) (# 130-098-235).
- (Optional) Further PepTivator – HT Peptide Pools, e.g., PepTivator AdV5 Hexon – HT (# 130-098-237).
- (Optional) Kits for the isolation and/or detection of cytokine-secreting T cells, e.g., IFN-γ Secretion Assay – Cell Enrichment and Detection Kit (PE), human (# 130-054-201) or TNF-α Secretion Assay – Cell Enrichment and Detection Kit (PE), human (# 130-091-269).
- (Optional) Antibodies or kits for intracellular cytokine staining, e.g., IFN-γ Antibody, anti-human, PE. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Cell surface staining of CD154 by using CD154 antibodies, human and CD40 pure – functional grade, human (# 130-094-133).
- (Optional) CytoStim™ (# 130-092-172, # 130-092-173) for restimulation of human T cells.
- (Optional) PepTivator CEF MHC Class I Plus – premium grade (# 130-098-426) as a peptide-specific positive control.

2.3 Recommendations for *in vitro* restimulation of antigen-specific T cells

▲ Before removing the plate from the aluminium pouch, warm up to room temperature. Remove the necessary amount of strips. Store the remaining ones at –20 °C in the tightly sealed aluminium pouch protected from humidity.

▲ CMV pp65-specific T cells are expected to be present only in certain individuals. Their frequency may be low compared to T cells with other specificities. The given protocol for *in vitro* T cell stimulation thus may only serve as a guideline.

▲ Always include a negative control (without antigen) in the experiment. As a positive control, a sample stimulated with, e.g., PepTivator CEF MHC Class I Plus or CytoStim may also be included.

1. Wash cells by adding medium, centrifuge at 300×g for 10 minutes. Aspirate supernatant.
2. Resuspend cells in culture medium at 10^7 cells per mL. Add 100 µL of the cell suspension to each well (10^6 cells/well). The final concentration of PepTivator CMV pp65 – HT in the cell suspension is 0.6 nmol of each peptide/mL.
3. Incubate cells at 37 °C and 5% CO_2.

   Cytokine Secretion Assay: Incubate cells for 3–6 hours.

   Cell surface staining of CD154: Add CD40 antibody pure – functional grade to the cell culture.

   Intracellular cytokine staining with antibodies or kits: Incubate cells for 2 hours, then add 1 µg/mL brefeldin A, and incubate for further 4 hours.
4. Collect cells carefully by using a cell scraper, or by pipetting up and down when working with smaller volumes. Rinse the dish with cold buffer. Check microscopically for any remaining cells, if necessary, rinse the dish again.

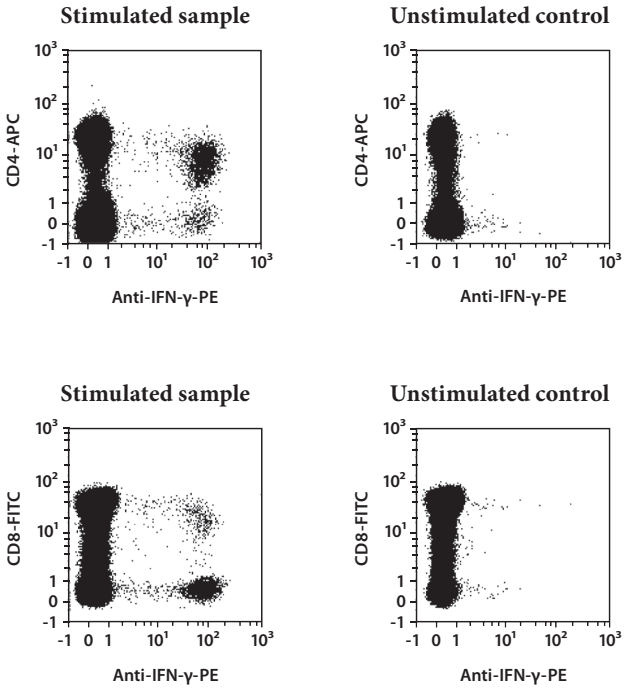
   To proceed with the Cytokine Secretion Assay or intracellular cytokine staining, please refer to the respective data sheet.

   ▲ **Note:** When preparing cells for **intracellular cytokine staining**, fixed cells may be stored at 2–8 °C for up to 1 week.

3. Examples

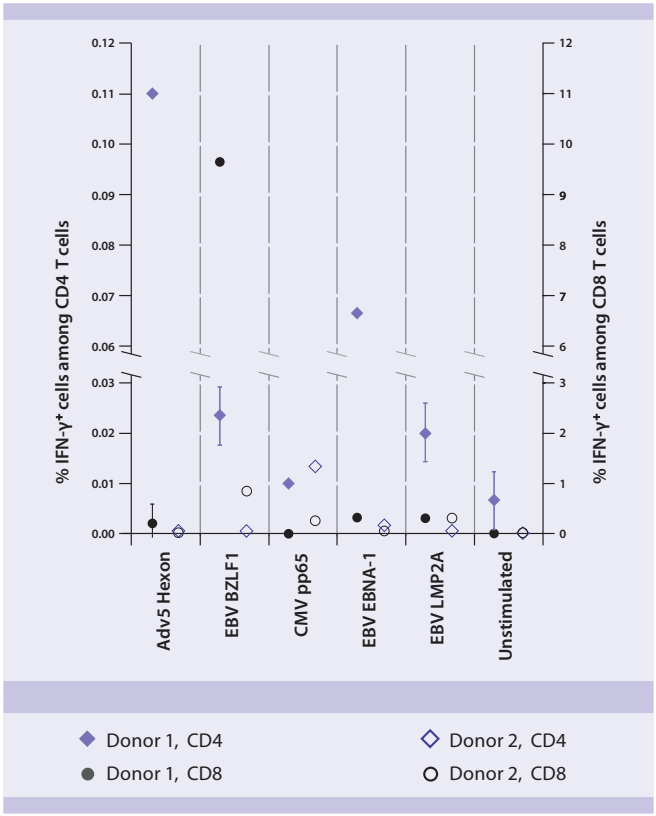
3.1 Detection of CMV pp65–specific T cells by intracellular staining with IFN-γ Antibody, anti-human, PE

From a CMV$^+$ donor 10^6 human PBMCs were restimulated for 6 hours with PepTivator CMV pp65 or without antigen. After 2 hours 1 µg/mL Brefeldin A was added. Cells were fixed, permeabilized, and CMV pp65–specific cells were intracellularly stained with Anti-IFN-γ-PE. T cells were counterstained for CD4 and CD8 expression. IFN-γ production of T cells is shown.



3.2 IFN-γ production upon stimulation with peptide pools

The following figure shows the production of IFN-γ by human $CD4^+$ or $CD8^+$ T cells after stimulation with different antigens using PepTivator Peptide Pools. Data from two different donors are shown (triplicates).



4. References

1. Pande, H. *et al.* (1984) Cloning and physical mapping of a gene fragment coding for a 64- kilodalton major late antigen of human cytomegalovirus. Proc. Natl. Acad. Sci. USA. 81: 4965–4969.
2. Kern, F. *et al.* (2002) Cytomegalovirus (CMV) phosphoprotein 65 makes a large contribution to shaping the T cell repertoire in CMV-exposed individuals. J. Infect. Dis. 185: 1709–1716.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

Legal notices