

PepTivator® CMV IE-1 – premium grade human

6 nmol/peptide
60 nmol/peptide

130-093-493
130-093-494

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1. Description

Components	6 nmol/peptide PepTivator® CMV IE-1 – premium grade or 60 nmol/peptide PepTivator CMV IE-1 – premium grade: Pool of lyophilized peptides, consisting mainly of 15-mer sequences with 11 amino acids (aa) overlap, covering the complete sequence of the IE-1 protein of human cytomegalovirus (CMV) strain AD169 (Swiss-Prot Acc. no. P13202).
Capacity	6 nmol (approximately 10 µg) per peptide for stimulation of up to 10 ⁸ total cells or 60 nmol (approximately 100 µg) per peptide for stimulation of up to 10 ⁹ total cells.
Product format	Lyophilized peptides containing stabilizer.
Purity	Each peptide >80%, peptides are individually purified by HPLC. Low endotoxin.
Storage	Store lyophilized product at –20 °C. The expiration date is indicated on the vial label.

This product contains no preservative and is sterile filtered; always handle under aseptic conditions.

1.1 Background information

Human Cytomegalovirus is a member of the herpesvirus group and belongs to the subfamily of beta-herpes viruses. 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.

IE-1 (72 kDa major immediate early protein), also known as UL123, is a non-structural protein and one of the first CMV antigens expressed in an infected cell. IE-1 predominantly induces a CD8⁺ T cell response.

The immunogenicity of the IE-1 protein has already been reported two decades ago.¹ To date it is known that IE-1-specific T cells occur in infected individuals at frequencies comparable to those of pp65-specific CD8 T cells.² Both CMV antigens, IE-1 and pp65 are now considered dominant T cell targets.

The correlation of the frequency of IE-1-specific CD8 T cells with protection from CMV disease has also been shown.³

The PepTivator® CMV IE-1 – premium grade is specially developed for efficient *in vitro* stimulation of IE-1-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.⁴ Stimulation of T cells with PepTivator CMV IE-1 – premium grade causes the secretion of effector cytokines and upregulation of activation markers, which then allows the detection or isolation of IE-1-specific T cells. Quantitative, phenotypical, or functional analysis of IE-1-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 CMV IE-1-specific CD4⁺ and CD8⁺ effector/memory T cells, for example, in PBMCs, by MACS® Cytokine Secretion Assays, intracellular cytokine staining, or other technologies.
- Isolation of viable CMV IE-1-specific CD4⁺ T cells with the CD154 MicroBead Kit.
- Isolation of viable CMV IE-1-specific CD4⁺ and CD8⁺ T cells using MACS Cytokine Secretion Assay – Cell Enrichment and Detection Kits or the CD137 MicroBead Kit, to be expanded for generation of T cell lines/clones for research on immunotherapy in CMV infection.
- Generation of CMV IE-1-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.

2. Recommendations for *in vitro* restimulation of antigen-specific T cells with PepTivator® CMV IE-1 – premium grade

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.

2.2 Reagent requirements

- Culture medium, e.g., RPMI 1640 (# 130-091-440) containing 5% human serum, e.g., autologous or AB serum.
▲ **Note:** Do not use BSA or FCS because of non-specific stimulation.
- (Optional) Cytokine Secretion Assay Kit. For additional reagent and instrument requirements refer to the data sheet of the respective Cytokine Secretion Assay.
- (Optional) Intracellular cytokine staining, e.g., with Anti-IFN-γ-PE (# 130-091-653). For additional reagent requirements refer to the respective data sheet. For more information on other fluorochrome-conjugates see www.miltenyibiotec.com.
- (Optional) Intracellular cytokine staining of activated CD4⁺ T cells by using, for example, the CD154/IFN-γ/CD4 Detection Kit (# 130-092-814).
- (Optional) CD154 MicroBead Kit (# 130-092-658). For details see the CD154 MicroBead Kit data sheet.
- (Optional) CD137 MicroBead Kit (# 130-093-476). For details see the CD137 MicroBead Kit data sheet.
- (Optional) CytoStim for restimulation of human T cells (# 130-092-172, # 130-092-173). For details see the CytoStim data sheet.
- (Optional) PepTivator CEF MHC Class I Plus – premium grade (# 130-098-426) as a peptide-specific positive control.

2.3 Recommendations for reconstitution of PepTivator® CMV IE-1 – premium grade

1. For reconstitution of the lyophilized peptide pool take the vial from –20 °C and warm-up to room temperature.
▲ **Note:** Do not open the vial by removing the rubber-stopper.
2. To dissolve the 6 nmol PepTivator® CMV IE-1 – premium grade fill a sterile syringe (0.5 mL) with 200 µL of sterile water. To dissolve the 60 nmol PepTivator® CMV IE-1 – premium grade fill a sterile syringe (5 mL) with 2 mL of sterile water.
3. Slowly inject the water with a sterile needle through the center of the rubber-stopper into the vial containing the lyophilized peptide pool.
4. Vortex the solution to completely dissolve the lyophilized peptide pool.
The concentration of the stock solution of PepTivator® CMV IE-1 – premium grade is 30 nmol (approximately 50 µg) of each peptide per mL.

5. Remove the rubber-stopper and aspirate the stock solution with a pipette.
6. To avoid repeated freeze-thaw cycles prepare working aliquots from the stock solution.
7. Store the working aliquots at –80 °C.

2.4 *In vitro* restimulation of antigen-specific T cells

▲ Always include a negative control (without antigen) in the experiment. A positive control (e.g. 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 10⁷ cells/mL. Plate cells in dishes at a density of 5×10⁶ cells/cm² (see 5. Appendix: Flask and dish sizes for *in vitro* T cell stimulation).
3. Mix the reconstituted PepTivator® Peptide Pool thoroughly. Add 20 µL of peptide pool stock solution per mL cell suspension. Mix carefully and incubate cells at 37 °C; 5% CO₂. The final concentration of peptide pool in the cell suspension is 0.6 nmol (approximately 1 µg) of each peptide/mL.

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

CD154 MicroBead Kit: Incubate cells for 4–16 hours.

CD137 MicroBead Kit: Incubate cells for 16–24 hours.

Intracellular cytokine staining antibodies or kits, e.g., CD154/IFN-γ/CD4 Detection Kit: 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.

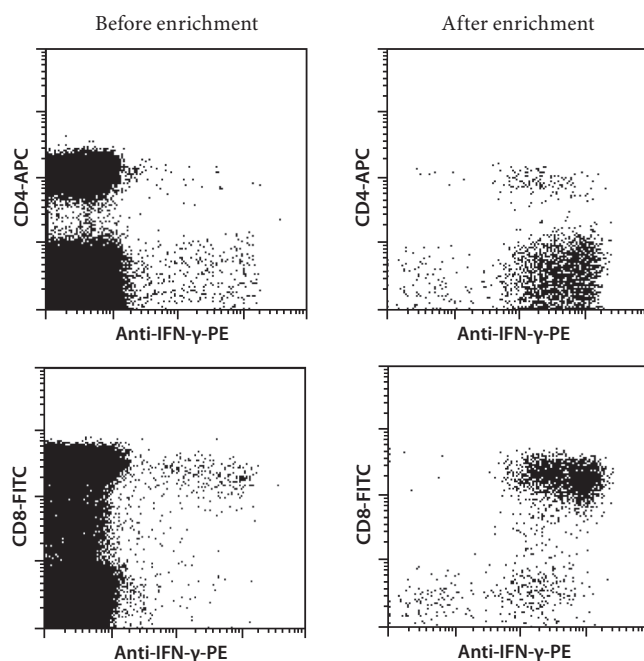
To proceed with the Cytokine Secretion Assay, the CD154 or CD137 MicroBead Kits, or intracellular cytokine staining, please refer to the respective data sheet.

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

3. Examples

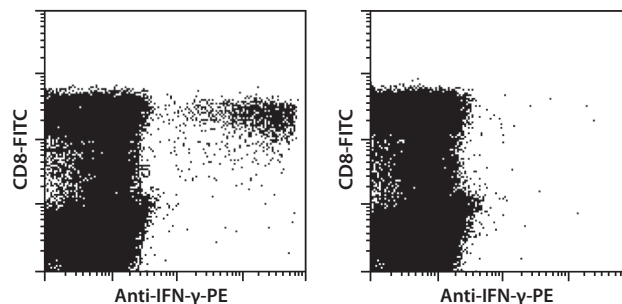
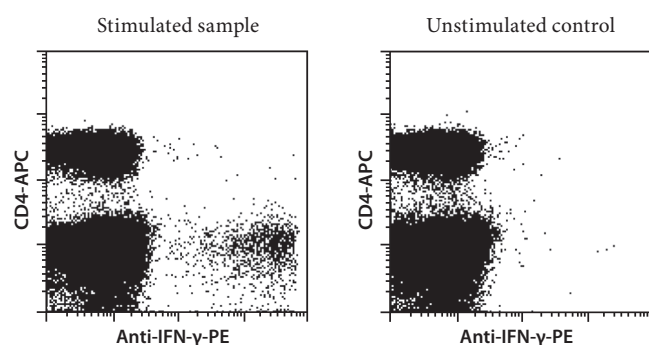
3.1 Detection and isolation of viable CMV IE-1-specific T cells using MACS® IFN-γ Secretion Assay – Cell Enrichment and Detection Kit (PE)

From a CMV⁺ donor 10⁷ human PBMCs were restimulated for 4 hours with 20 μL/mL of reconstituted PepTivator CMV IE-1 – premium grade. CMV IE-1-specific cells were stained and magnetically enriched according to their secretion of IFN-γ using the IFN-γ Secretion Assay – Cell Enrichment and Detection Kit (PE) (# 130-054-201). T cells were counterstained for CD4 and CD8 expression. Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence. IFN-γ secretion of viable lymphocytes is shown.



3.2 Detection of CMV IE-1-specific T cells by intracellular staining with Anti-IFN-γ-PE

From a CMV⁺ donor 10⁶ human PBMCs were restimulated for 6 hours with 20 μL/mL of reconstituted PepTivator CMV IE-1 – premium grade or without antigen. After 2 hours 1 μg/mL Brefeldin A was added. Cells were fixed, permeabilized, and CMV IE-1-specific cells were intracellularly stained with Anti-IFN-γ-PE (# 130-091-653). T cells were counterstained for CD4 and CD8 expression. IFN-γ production of lymphocytes is shown.



4. References

1. Borysiewicz, L. K. *et al.* (1988) Human cytomegalovirus-specific cytotoxic T cells. Relative frequency of stage-specific CTL recognizing the 72-kD immediate early protein and glycoprotein B expressed by recombinant vaccinia viruses. *J. Exp. Med.* 168: 919–931.
2. Kern, F. *et al.* (1999) Target structures of the CD8⁺ T cell response to human cytomegalovirus: the 72-kilodalton major immediate-early protein revisited. *J. Virol.* 73: 8179–8184.
3. Bunde, T. *et al.* (2005) Protection from cytomegalovirus after transplantation is correlated with immediate early 1-specific CD8 T cells. *J. Exp. Med.* 201: 1031–1036.
4. Kiecker, F. *et al.* (2004) Analysis of antigen-specific T-cell responses with synthetic peptides — what kind of peptide for which purpose? *Hum. Immunol.* 65: 523–536.

5. Appendix: Flask and dish sizes for *in vitro* T cell stimulation

For *in vitro* T cell stimulation (see 2.4) the cells should be resuspended in culture medium, containing 5% of human serum, at a dilution of 10⁷ cells/mL. The cells should be plated at a density of 5×10⁶ cells/cm². Both the dilution and the cell density are important to assure optimum stimulation.

The following table lists culture plate, dish and flask sizes suitable for different cell numbers. It also indicates the appropriate amount of medium to add.

Total cell number	Medium volume to add	Culture plate	Well diameter
0.15×10 ⁷	0.15 mL	96 well	0.64 cm
0.50×10 ⁷	0.50 mL	48 well	1.13 cm
1.00×10 ⁷	1.00 mL	24 well	1.60 cm
2.00×10 ⁷	2.00 mL	12 well	2.26 cm
5.00×10 ⁷	5.00 mL	6 well	3.50 cm
Total cell number	Medium volume to add	Culture dish	Dish diameter
4.5×10 ⁷	4.5 mL	small	3.5 cm
10.0×10 ⁷	10.0 mL	medium	6 cm
25.0×10 ⁷	25.0 mL	large	10 cm
50.0×10 ⁷	50.0 mL	extra large	15 cm
Total cell number	Medium volume to add	Culture flask	Growth area
12×10 ⁷	12 mL	50 mL	25 cm ²
40×10 ⁷	40 mL	250 mL	75 cm ²
80×10 ⁷	80 mL	720 mL	162 cm ²
120×10 ⁷	120 mL	900 mL	225 cm ²

Refer to www.miltenyibiotec.com for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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