Miltenyi Biotec

CMV pp65 – Recombinant Protein

human cytomegalovirus

200 µL	130-091-824
2×1 mL	130-091-823

Contents

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

1. Description

Components	**200 µL CMV pp65 – Recombinant Protein** or **2×1 mL CMV pp65 – Recombinant Protein:** full-length protein (Swiss-Prot Acc. no. P06725)
Capacity	200 µL for stimulation of up to 2×10^8 total cells, or 2× 1 mL for stimulation of up to 2×10^9 total cells.
Product format	The recombinant protein is supplied in a solution of 50 mM sodium dihydrogenphosphate, 300 mM NaCl, and 4 M urea.
Purity	>95% as determined by SDS-PAGE analysis. Low endotoxin.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

Human cytomegalovirus (CMV) is a member of the herpes virus 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.

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. pp65 is an immunodominant target of $CD4^+$ as well as $CD8^+$ T cell responses to CMV.[2] pp65-specific T cells predominantly produce cytokines like IFN-γ, IL-2, and TNF-α.

CMV pp65 – Recombinant Protein is specially developed for efficient *in vitro* stimulation of pp65-specific $CD4^+$ and $CD8^+$ T cells, for example, causing secretion of effector cytokines. The produced cytokines then allow detection or isolation of pp65-specific T cells. Quantitative, phenotypical, or functional analysis of pp65-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 pp65-specific $CD4^+$ and $CD8^+$ effector/memory T cells, e.g., in PBMCs, by MACS® Cytokine Secretion Assays, intracellular cytokine staining, or other technologies.
- Isolation of viable pp65-specific $CD4^+$ and $CD8^+$ T cells using MACS Cytokine Secretion Assay – Cell Enrichment and Detection Kits, the CD154 MicroBead Kit, or the CD137 MicroBead Kit, for example, to be expanded for generation of T cell lines/clones for research on immunotherapy in CMV infection.
- Generation of pp65-specific $CD4^+$ and $CD8^+$ effector/memory T cells from naive T cell populations, for example, for research on immunotherapy and vaccination.
- Pulsing of antigen-presenting cells, for example, for research on dendritic cell vaccination.
- Research on antigen delivery by antigen-presenting cells.

2. Recommendations for *in vitro* restimulation of pp65-specific T cells with CMV pp65 – Recombinant Protein

2.1 Sample preparation

For induction of cytokine secretion by pp65-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 aspirate supernatant.

▲ **Note:** PBMCs may be stored overnight. The cells should be resuspended and incubated in culture medium as described in 2.3 steps 1–3., but without addition of antigen. The antigen is then added to the culture on the next day.

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2.2 Reagent requirements

- Culture medium, for example, RPMI 1640 (# 130-091-440) containing 5% human serum, e.g., autologous or AB serum (do not use BSA or FBS 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, for example, with Anti-IFN-γ-FITC (# 130-091-641). For additional reagent requirements refer to the respective data sheet.
- (Optional) CD154 Cytokine Detection Kits, CD154 antibodies, or CD154 MicroBead Kit (# 130-092-658). For more information refer to the respective data sheets.
- (Optional) CD137 MicroBead Kit (# 130-093-476) and CD137-PE (# 130-093-475). For more information refer to the respective data sheets.
- (Optional) CytoStim™ for restimulation of human T cells (# 130-092-172, # 130-092-173). For details refer to the CytoStim data sheet.

2.3 *In vitro* restimulation of pp65-specific T cells

▲ Always include a negative control in the experiment. The sample should be treated in exactly the same manner as the stimulated sample but without the addition of stimulants.

▲ A positive control, such as a sample stimulated with CytoStim, may also be included.

▲ Do not use media containing non-human proteins, such as BSA or FBS, because of non-specific stimulation.

1. Wash cells by adding medium and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cells in culture medium at 10^7 cells/mL. Plate cells in dishes at a density of 5×10^6 cells/cm² (refer to 5. Appendix: Flask and dish sizes for *in vitro* stimulation of T cells).
3. Mix CMV pp65 – Recombinant Protein thoroughly. Add 10 µL of CMV pp65 – Recombinant Protein per mL cell suspension. Mix carefully and incubate cells at 37 °C; 5–7% CO_2.

   Cytokine Secretion Assay: Incubate cells for 4–16 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 pipetting up and down when working with smaller volumes or by using a cell scraper. Rinse the dish with cold buffer. Check microscopically for any remaining cells, if necessary, rinse the dish again.

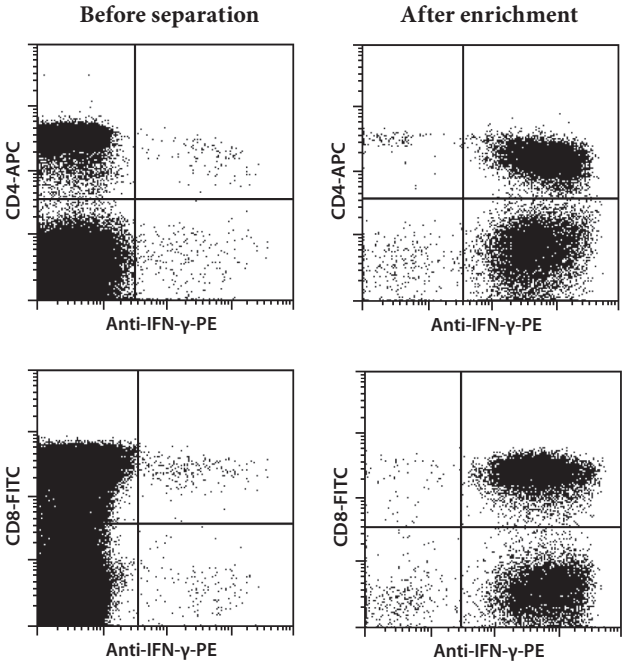
   To proceed with the Cytokine Secretion Assay, the CD154 or CD137 MicroBead Kits, or intracellular cytokine staining, please refer to the respective data sheet.

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

3. Examples

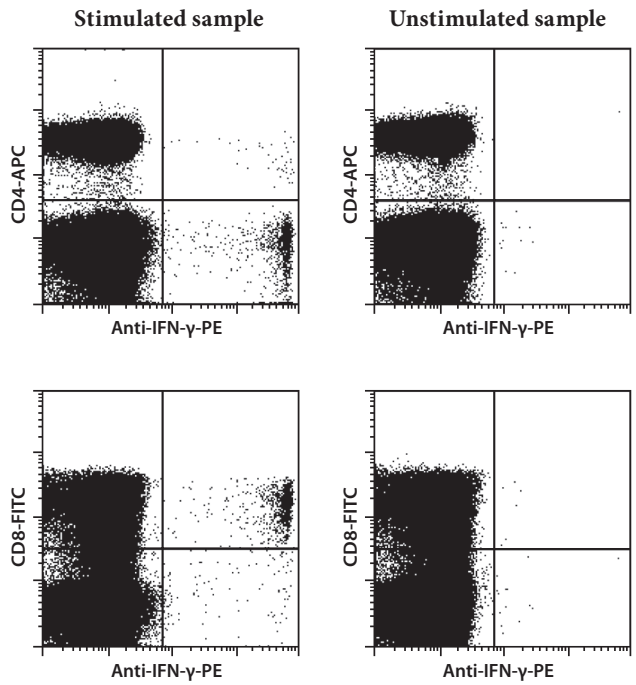
3.1 Detection and isolation of viable pp65-specific T cells using MACS® IFN-γ Secretion Assay

10^7 human PBMCs of a CMV⁺ donor were restimulated with CMV pp65 – Recombinant Protein. pp65-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 (# 130-054-201). T cells were counterstained for CD4 and CD8 expression. IFN-γ-secretion of viable lymphocytes is shown.



3.2 Detection of pp65-specific T cells by intracellular staining of IFN-γ

10^6 human PBMCs of a CMV⁺ donor were restimulated with CMV pp65 – Recombinant Protein or without antigen, after 2 hours 1 µg/mL of brefeldin A was added. Cells were fixed, permeabilized, and pp65-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. 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.

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

For *in vitro* stimulation of T cells (refer to 2.3) the cells should be resuspended in culture medium, containing 5% of human serum, at a dilution of 10^7 cells/mL. The cells should be plated at a density of 5×10^6 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 cm²
40×10^7	40 mL	250 mL	75 cm²
80×10^7	80 mL	720 mL	162 cm²
120×10^7	120 mL	900 mL	225 cm²

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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