Miltenyi Biotec

hCMV-pp65 Antigen Delivery Reagent

Order no. 130-095-406

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1. Description

Components	200 µL hCMV-pp65 Antigen Delivery Reagent: fluorescein isothiocyanate (FITC)-labeled anti-biotin monoclonal antibodies (isotype: mouse IgG1) conjugated to PepTivator® CMV pp65 covalently coupled to polyamidoamine (PAMAM) dendrimer.
Capacity	20 tests or up to 2×10^7 total cells.
Product format	Antibodies are supplied in buffer containing stabilizer. Endotoxin levels have been tested and do not exceed >0.1 EU/mL labeling concentration.
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

Cytomegalovirus (CMV) is the best studied virus in humans. More than half of human adults are infected with CMV. The pp65 CMV envelope protein is a dominant antigen and contains a vast amount of well-defined T cell epitopes.[1,2] Therefore, pp65 is a widely used model system to study human T cell responses and human antigen-presenting cell (APC) function.

The hCMV-pp65 Antigen Delivery Reagent has been developed for specific targeting of pp65 CMV antigen to antigen uptake receptors on human APCs. Such targeted delivery of antigen allows the analysis of antigen uptake, processing, and antigen presenting capability of targeted APCs.[3–5] The reagent consists of pp65 peptides covalently coupled to a dendrimer and a monoclonal, anti-biotin-FITC conjugate. Using a biotinylated primary antibody, the antigen can be specifically targeted to any surface molecule of the relevant APCs. This allows the identification and functional characterization of antigen uptake receptors, as well as research on vaccine strategies.

1.2 Applications

The hCMV-pp65 Antigen Delivery Reagent is well suited for analysis of

- antigen uptake by antigen-presenting cells, i.e., DCs, macrophages, and B cells.
- antigen processing and intracellular trafficking of endocytotic receptors.
- cross presentation of antigens.
- protocols for antigen delivery for DC vaccination.

1.3 Reagent requirements

- Buffer: Prepare a sterile solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% AB serum, and 2 mM EDTA. Keep buffer cold (2–8 °C).

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD).

  ▲ **Note:** EDTA inhibits T cell stimulation. Cells should be washed in ETDA-free buffer or medium before co-culture with T cells.

  ▲ **Note:** Staining of some antigen uptake receptors, e.g., members of the c-type lectin family, require the presence of Ca^{2+} ions. Please use buffers recommended by the manufacturer for staining of those receptors with monoclonal antibodies.

- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor–mediated antibody labeling.
- (Optional) CD304 (BDCA-4/Neuropilin-1) MicroBead Kit, human (# 130-090-532) for the isolation of plasmacytoid dendritic cells.
- (Optional) Memory $CD4^+$ T Cell Isolation Kit (# 130-091-893) for the isolation of plasmacytoid dendritic cells.
- (Optional) CD36-Biotin, human (# 130-095-478). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Anti-Fluorochrome MicroBeads, e.g., Anti-FITC MicroBeads (# 130-048-701), Anti-PE MicroBeads (# 130-048-801), or Anti-APC MicroBeads (# 130-090-855) for biotin-free isolation of APCs.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Pre-Separation Filters, 30 µm (# 130-041-407) to remove cell clumps.

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2. Protocol

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation, for example, using Ficoll-Paque™.

▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully aspirate supernatant. Repeat washing step.

When working with tissues or lysed blood, prepare a single-cell suspension using standard methods.

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

▲ Dead cells may bind non-specifically to MACS® MicroBeads. To remove dead cells, we recommend using density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101)

2.2 *In vitro* targeting using hCMV-pp65 Antigen Delivery Reagent

▲ The hCMV-pp65 Antigen Delivery Reagent contains anti-biotin antibodies. It is recommended to avoid biotin-labeled antibodies for enrichment of antigen-presenting cells. Therefore, use direct MicroBeads, flow sorting, or indirect isolation via Anti-Fluorochrome MicroBeads to circumvent cross-targeting of antigen.

▲ Method in brief: Isolated antigen-presenting cells are labeled with a biotin-conjugated antibody, which is specific for a cell surface receptor of interest. Cells are then labeled with the hCMV-pp65 Antigen Delivery Reagent for receptor-mediated targeting of hCMV-pp65 to these cells.

▲ Volumes given below are for **up to 10^6** nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^6 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ For accurate analysis of antigen targeting, a negative control sample should always be included, for example, by omitting the antigen uptake receptor–specific biotin-conjugated antibody.

▲ Positive control, such as a sample labeled with CD36-Biotin may also be included. For details refer to the respective data sheet.

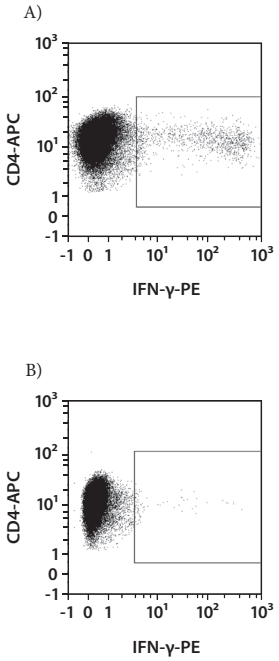
▲ Work under sterile conditions throughout the whole experiment.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes at 4 °C. Aspirate supernatant completely.
3. Label cells with biotinylated antibody at time and titer recommended by the manfacturer. Typically, labeling for 10 minutes is sufficient.
   ▲ **Note:** The biotinylated antibody should be used at its optimal titer, i.e., with optimal labeling intensity and no background labeling.
4. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge for 10 minutes at 300×g and 4 °C.
   ▲ **Note:** The optimal relative centrifugal force (RCF) and centrifugation time may be different depending on the cell sample.
5. Resuspend up to 10^6 nucleated cells per 100 µL of cold buffer.
6. Add 10 µL of the hCMV-pp65 Antigen Delivery Reagent.
7. Mix well and incubate for 10 minutes on ice.
   ▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
8. Wash cells by adding 1–2 mL of buffer or medium and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
   ▲ **Note:** EDTA inhibits T cell stimulation. Cells should be washed in ETDA-free buffer or medium before co-culture with T cells.
9. For subsequent analysis by flow cytometry resuspend cell pellet in a suitable amount of buffer or for culture in medium.

   Recommendation: The CMV-antigen–presenting capabilities of targeted APCs can be analyzed by co-culture with autologous CMV-specific T cells using proliferation or cytokine production as read out.

3. Examples for use of the hCMV-pp65 Antigen Delivery Reagent

Plasmacytoid dendritic cells (PDCs) were isolated using BDCA-4 MicroBeads. PDCs were labeled with CD36-Biotin (A) or with a biotin-conjugated isotype control (B) followed by labeling with the hCMV-pp65 Antigen Delivery Reagent. Cells were cultured in the presence of CpG ODN for 24 hours that induces PDC maturation via toll-like receptor 9. Subsequently, PDCs were co-cultured with autologous memory effector $CD4^+$ T cells at a ratio of 1:5 (PDC:T cell) for 20 hours by adding brefeldin A in the last 4 hours. Cells were fixed, permeabilized, and analyzed for intracellular IFN-γ by flow cytometry.[6]



4. References

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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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