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1. Description

This product is for research use only.

Components	1×96-well plate, consisting of 12 strips of 8 wells Pool of 43 lyophilized MHC class I and class II restricted peptides derived from 13 different proteins from Epstein-Barr virus (EBV).
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 >90% (HPLC peak area at 220 nm wavelength), 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.

EBV is a human γ -herpesvirus with B cell growth-transforming ability and lymphomagenic potential. More than 90 % of human adults are infected with EBV. In healthy individuals, EBV typically establishes a persistent latent infection in which the virus can be detected in resting, non-proliferating peripheral B lymphocytes. T cells are essential for the control of the outgrowth of EBV-infected B cells and several EBV proteins have been shown to be immunogenic targets in EBV-associated malignancies, such as nasopharyngeal carcinoma and Hodgkin's lymphoma.

The PepTivator EBV Consensus pool contains 43 peptides of 8–20 aa in length. 32 of these peptides are MHC class I and 11 peptides

are MHC class II restricted. The sequences of these peptides were derived from the following 15 different lytic and latent EBV-proteins: LMP2a; BRLF1; BMLF1 (EB2); BNLF1 (LMP1); BERF3 (EBNA6); BERF1 (EBNA3); BERF2 (EBNA4); BALF2 (DNBI); BMRF1; BZLF1; BNRF1 (MTP); EBNA1; BLLF1 (gp350); BXLF2; EBNA2. The peptides are restricted for the following HLA-molecules: HLA-A*02, -A*03, -A*11, -A*24, -A*26, -B*07, -B*08, -B*15, -B*18, -B*27, -B*35, -B*40, -B*4402, -DR.

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.

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 CMV pp65 (# 130-097-727).
- (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.

▲ EBV Consensus-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 10⁷ cells per mL. Add 100 µL of the cell suspension to each well (10⁶ cells/well). The final concentration of PepTivator EBV Consensus (HT) – premium grade in the cell suspension is 0.6 nmol of each peptide/mL.
3. Incubate cells at 37 °C and 5% CO₂.

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.

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.

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