Miltenyi Biotec

PepTivator® CEF MHC Class I Plus – premium grade

6 nmol/peptide 130-098-426

Contents

1. Description

Components	**6 nmol/peptide PepTivator® CEF MHC Class I Plus – premium grade** Pool of 32 lyophilized peptides.
Capacity	6 nmol (approximately 10 µg) per peptide for stimulation of up to 10^8 total cells.
Product format	Lyophilized peptides containing stabilizer.
Purity	Each peptide >95% (HPLC peak area at 220 nm), 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

The PepTivator® CEF MHC Class 1 Plus consists of 32 MHC-1–specific peptides of 8–12 aa in length. These sequences were derived from cytomegalovirus (HCMV), Epstein-Barr virus (EBV), and influenza virus and have been shown to elicit interferon-gamma release from $CD8^+$ T cells in most of all individuals.[1,2] For peptide sequences and HLA restrictions refer to the following table. HLA-allele data according to www.iedb.org.

Sequence	Source	Protein	HLA allele
VSDGGPNLY	Influenza A	RNA polymerase	HLA-A1
CTELKLSDY	Influenza A	Nucleoprotein	HLA-A1
GILGFVFTL	Influenza A	Matrix Protein	HLA-A2
FMYSDFHFI	Influenza A	Polymerase PA	HLA-A2
CLGGLLTMV	EBV	LMP2A	HLA-A2
GLCTLVAML	EBV	BMLF1	HLA-A2
NLVPMVATV	HCMV	pp65	HLA-A0201
RVLSFIKGTK	Influenza A	Nucleoprotein	HLA-A3
ILRGSVAHK	Influenza A	Nucleoprotein	HLA-A3
RVRAYTYSK	EBV	BRLF1	HLA-A3
RLRAEAQVK	EBV	EBNA 3A	HLA-A3
SIIPSGPLK	Influenza A	Matrix Protein	HLA-A3/A11/A68
AVFDRKSDAK	EBV	EBNA-3B	HLA-A11
IVTDFSVIK	EBV	EBNA-3B	HLA-A11
ATIGTAMYK	EBV	BRLF1	HLA-A11
DYCNVLNKEF	EBV	BRLF1	HLA-A24
KTGGPIYKR	Influenza A	Nucleoprotein	HLA-A68
LPFDKTTVM	Influenza A	Nucleoprotein	HLA-B7
RPPIFIRRL	EBV	EBNA 3A	HLA-B7
TPRVTGGGAM	HCMV	pp65	HLA-B7
ELRSRYWAI	Influenza A	Nucleoprotein	HLA-B8
RAKFKQLL	EBV	BZLF1	HLA-B8
FLRGRAYGL	EBV	EBNA 3A	HLA-B8
QAKWRLQTL	EBV	EBNA 3A	HLA-B8
EFFWDANDIY	HCMV	pp65	HLA-B12/B44
SDEEEAIVAYTL	HCMV	IE-1	HLA-B18
SRYWAIRTR	Influenza A	Nucleoprotein	HLA-B27
ASCMGLIY	Influenza A	Matrix Protein	HLA-B27
RRIYDLIEL	EBV	EBNA 3C	HLA-B27
YPLHEQHGM	EBV	EBNA 3A	HLA-B35
IPSINVHHY	HCMV	pp65	HLA-B35
EENLLDFVRF	EBV	EBNA 3C	HLA-B44

1.2 Applications

- The PepTivator CEF MHC Class 1 Plus is recommended as a peptide-specific positve control for the detection of $CD8^+$ T cells in human PBMC. This peptide set is used by the NIH. For more information refer to the NIH AIDS Research and Reference Reagent Program.
- Detection and analysis of virus-specific $CD8^+$ effector/memory T cells, e.g. in PBMCs, by MACS® Cytokine Secretion Assays, intracellular cytokine staining, or other technologies.

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2. Recommendations for *in vitro* restimulation of virus–specific T cells with PepTivator® Peptide Pools

2.1 Cell preparation

For induction of cytokine secretion by virus–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 (# 130-091-440) 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.

- (Optional) Cytokine Secretion Assay Kit.
- (Optional) Antibodies or kits for intracellular cytokine staining, e.g., IFN-γ Antibody-PE or CD154/IFN-γ/CD4 Detection Kit (# 130-092-814). For more information on other fluorochrome-conjugates refer to www.miltenyibiotec.com/antibodies.
- (Optional) CD154 MicroBead Kit (# 130-092-658) or CD137 MicroBead Kit (# 130-093-476).

2.3 Recommendations for reconstitution of PepTivator® Peptide Pools

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 plug.

2. To dissolve the PepTivator® Peptide Pool fill a sterile syringe (0.5 mL) with 200 µL of sterile water.
3. Slowly inject the water with a sterile needle through the center of the rubber plug 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 Peptides is 30 nmol (approximately 50 µg) of each peptide per mL.
5. Remove the rubber plug 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 Recommendations for *in vitro* restimulation of virus–specific T cells

▲ Magnetic enrichment of stimulated virus–specific T cells according to cytokine secretion using the MACS Secretion Assay Technology or according to expression of activation marker, e.g. CD154, will enhance the sensitivity of the assay.

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/mL. Plate cells in dishes at a density of 5×10^6 cells/cm² (refer to 3. Appendix: Flask and dish sizes for *in vitro* T cell stimulation).
3. Mix the reconstituted PepTivator Peptide Pool thoroughly. Add 20 µL of PepTivator stock solution per mL cell suspension. Mix carefully and incubate cells at 37 °C and 5% CO_2.

   The final concentration of PepTivator 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.

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

For *in vitro* T cell stimulation (refer to 2.4) 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

4. References

1. Currier, J.R. *et al.* (2002) A panel of MHC class I restricted viral peptides for use as a quality control for vaccine trial ELISPOT assays. J. Immunol. Methods 260(1–2): 157–172.
2. Mwau, M. *et al.* (2002) Design and validation of an enzyme-linked immunospot assay for use in clinical trials of candidate HIV vaccines. AIDS Res. Hum. Retroviruses 18(9): 611–618.

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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