Miltenyi Biotec

PepTivator® Melan-A/MART-1 – premium grade

human

6 nmol/peptide	130-094-597
60 nmol/peptide	130-094-477

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1. Description

Components	**6 nmol/peptide PepTivator® Melan-A/MART-1 – premium grade** or **60 nmol/peptide PepTivator® Melan-A/MART-1 – premium grade:** Pool of lyophilized peptides, consisting mainly of 15-mer sequences with 11 amino acids overlap, covering the complete sequence of the human Melan-A/MART-1 protein (Swiss-Prot Acc. no. Q16655).
Capacity	6 nmol (approximately 10 µg) per peptide for the stimulation of up to 10^8 total cells or 60 nmol (approximately 100 µg) per peptide for the 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

Melanoma antigen recognized by T-cells (MART-1) belongs to the tumor-associated antigens of the group of differentiation antigens. Its expression is restricted to normal melanocytes but it is also present in melanoma. $CD4^+$ and $CD8^+$ T lymphocytes recognizing Melan-A/MART-1 have been identified in melanoma patients. Therefore Melan-A/MART-1 represents a potential target for immunotherapy of melanoma.[1]

The PepTivator® Melan-A/MART-1 – premium grade has been specially developed for efficient *in vitro* stimulation of Melan-A/MART-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. Stimulation of T cells with PepTivator Melan-A/MART-1 – premium grade causes the secretion of effector cytokines and upregulation of activation markers, which then allows the detection or isolation of Melan-A/MART-1–specific T cells. Quantitative, phenotypical, or functional analysis of Melan-A/MART-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 Melan-A/MART-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 Melan-A/MART-1–specific $CD4^+$ T cells with the CD154 MicroBead Kit.
- Isolation of viable Melan-A/MART-1–specific $CD4^+$ and $CD8^+$ T cells using MACS Cytokine Secretion Assay – Cell Enrichment and Detection Kits or the CD137 MicroBead Kit for *in vitro* generation of T cell lines/clones for research on tumor immunotherapy.
- Generation of Melan-A/MART-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, for example, dendritic cell vaccination.

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140-002-682.04

2. Recommendations for *in vitro* restimulation of Melan-A/MART-1–specific T cells with PepTivator® Melan-A/MART-1 – premium grade

2.1 Cell preparation

For induction of cytokine secretion by Melan-A/MART-1–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., Anti-IFN-γ-PE (# 130-091-653) or the CD154/IFN-γ/CD4 Detection Kit (# 130-092-814). For more information on other fluorochrome-conjugates refer to www.miltenyibiotec.com.
- (Optional) CD154 MicroBead Kit (# 130-092-658) or CD137 MicroBead Kit (# 130-093-476).
- (Optional) CytoStim for restimulation of human T cells (# 130-092-172, # 130-092-173).
- (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® Melan-A/MART-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® Melan-A/MART-1 – premium grade fill a sterile syringe (0.5 mL) with 200 µL of sterile water. To dissolve the 60 nmol PepTivator Melan-A/MART-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 Melan-A/MART-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 Recommendations for *in vitro* restimulation of Melan-A/MART-1–specific T cells

▲ Always include a negative control (without antigen) in the experiment. A positive control (e.g. a sample stimulated with 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 10^7 cells/mL. Plate cells in dishes at a density of 5×10^6 cells/cm² (refer to 4. Appendix: Flask and dish sizes for *in vitro* T cell stimulation).
3. Mix the reconstituted PepTivator Melan-A/MART-1 – premium grade thoroughly. Add 20 µL of PepTivator Melan-A/MART-1 – premium grade stock solution per mL cell suspension. Mix carefully and incubate cells at 37 °C; 5–7% CO_2.

   The final concentration of PepTivator Melan-A/MART-1 – premium grade 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. Reference

1. Boon, T. *et al.* (2006) Human T cell responses against melanoma. Annu. Rev. Immunol. 24: 175–208.

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140-002-682.04

4. 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

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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