Miltenyi Biotec

PepTivator® RAS G12D – premium grade

human

6 nmol/peptide 130-133-874
60 nmol/peptide 130-133-878

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1. Description

This product is for research use only.

Components	**6 nmol/peptide PepTivator RAS G12D – premium grade, human** or **60 nmol/peptide PepTivator RAS G12D – premium grade, human** Pool of lyophilized peptides, covering the sequence [MTEYKLVVVGA**D**GVGKSALTIQLIQN] with the single point mutation G12D. The pool contains every 9-mer, 10-mer, and 15-mer peptide of the RAS G12D protein that has the mutated amino acid at position 12, and each peptide is shifted by one amino acid compared to its predecessor.
Capacity	6 nmol (approximately 10 µg) per peptide for stimulation of up to 10^8 total cells or 60 nmol (approximately 100 µg) per peptide for 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 preservatives and is sterile filtered; always handle under aseptic conditions.

1.1 Background information

RAS or KRAS (Kirsten rat sarcoma virus; UniProtKB Acc. no P0 1116), belongs to a family of proteins that are ubiquitously expressed in all animal cell types and is composed of 189 amino acids (aa). It is involved in cell growth, differentiation, and survival. The mutations in RAS genes, here G12D, can lead to different cancer formation acting as oncogenes. PepTivator RAS G12D covers the above mentioned mutation in all three isoforms of RAS: K-Ras, N-Ras, and H-Ras. The RAS mutations are found within approximately 20–30% of all human cancers. Cancers with very high RAS mutational burden include pancreatic cancer (90%), colorectal cancer (55%), and non-small cell lung cancer (30%).

The PepTivator RAS G12D – premium grade has been specially developed for efficient *in vitro* stimulation of RAS G12D–specific $CD4^+$ and $CD8^+$ T cells, as peptides of 15-, 10-, and 9 amino acids in length represent an optimal solution for stimulating both $CD4^+$ and $CD8^+$ T cells in various applications, as well as providing broadest coverage of potential HLA alleles. Stimulation of T cells with this PepTivator Peptide Pool causes the secretion of effector cytokines and upregulation of activation markers, which then allows the detection or isolation of RAS G12D–specific T cells. Quantitative, phenotypical, or functional analysis of T cell immunity can provide important information on the course of immune responses in patients suffering from cancer or healthy 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.
- Isolation of viable antigen-specific $CD4^+$ T cells with the CD154 MicroBead Kit, human.
- Isolation of viable antigen-specific $CD4^+$ and $CD8^+$ T cells using MACS Cytokine Secretion Assay – Cell Enrichment and Detection Kits or the CD137 MicroBead Kit, human for *in vitro* generation of T cell lines/clones.
- 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.

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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.

- (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) CD154 MicroBead Kit, human (# 130-092-658) or CD137 MicroBead Kit, human (# 130-093-476).
- (Optional) CytoStim™ (# 130-092-172, # 130-092-173) for restimulation of human T cells.
- (Optional) PepTivator Negative Control – premium grade (# 130-131-610, # 130-131-612) as a matrix-specific control reagent.
- (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 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 6 nmol PepTivator Peptide Pool fill a sterile syringe (0.5 mL) with 200 µL of sterile water. To dissolve the 60 nmol PepTivator Peptide Pool 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 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 antigen-specific T cells

▲ RAS G12D–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.

▲ Magnetic enrichment of stimulated antigen-specific T cells according to cytokine secretion, e.g. IL-17, using the MACS Secretion Assay technology or according to expression of activation markers, e.g. CD154, will enhance the sensitivity to detect rare cells.

▲ 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 10^7 cells per 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 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 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 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, 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 optimal 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 for local Miltenyi Biotec Technical Support contact information.

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