

# PepTivator® NY-ESO-1 – premium grade human

6 nmol/peptide  
60 nmol/peptide

130-095-380  
130-095-381

## Contents

1. Description
  - 1.1 Background information
  - 1.2 Applications
2. Recommendations for *in vitro* restimulation of NY-ESO-1-specific T cells with PepTivator® NY-ESO-1 – premium grade
  - 2.1 Cell preparation
  - 2.2 Reagent requirements
  - 2.3 Recommendations for reconstitution of PepTivator® NY-ESO-1 – premium grade
  - 2.4 Recommendations for *in vitro* restimulation of NY-ESO-1-specific T cells
3. Reference
4. Appendix: Flask and dish sizes for *in vitro* T cell stimulation

## 1. Description

<b>Components</b>	6 nmol/peptide PepTivator® NY-ESO-1 – premium grade or 60 nmol/peptide PepTivator® NY-ESO-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 NY-ESO-1 protein (Swiss-Prot Acc. no. P78358).
<b>Capacity</b>	6 nmol (approximately 10 µg) per peptide for the stimulation of up to 10 <sup>8</sup> total cells or 60 nmol (approximately 100 µg) per peptide for the stimulation of up to 10 <sup>9</sup> total cells.
<b>Product format</b>	Lyophilized peptides containing stabilizer.
<b>Purity</b>	Each peptide >80%, peptides are individually purified by HPLC. Low endotoxin.
<b>Storage</b>	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

NY-ESO-1 belongs to the cancer-testis family of antigens. It is highly expressed in male germ cells but generally not in other normal tissues. Aberrant expression is found in a variable proportion of a wide range of different cancer types. Patients with advanced NY-ESO-1-expressing tumors frequently develop humoral and cellular immunity to NY-ESO-1.<sup>1</sup> NY-ESO is described to be an attractive antigen to monitor anti-tumor immune responses and to be used for immunotherapeutic interventions.

The PepTivator® NY-ESO-1 – premium grade has been specially developed for efficient *in vitro* stimulation of NY-ESO-1-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells, as peptides of 15 amino acid length with 11 amino acid overlap represent the optimized solution for stimulating both CD4<sup>+</sup> and CD8<sup>+</sup> T cells in various applications. Stimulation of T cells with PepTivator NY-ESO-1 – premium grade causes the secretion of effector cytokines and upregulation of activation markers, which then allows the detection or isolation of NY-ESO-1-specific T cells. Quantitative, phenotypical, or functional analysis of NY-ESO-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 NY-ESO-1-specific CD4<sup>+</sup> and CD8<sup>+</sup> effector/memory T cells, for example, in PBMCs, by MACS® Cytokine Secretion Assays, intracellular cytokine staining, or other technologies.
- Isolation of viable NY-ESO-1-specific CD4<sup>+</sup> T cells with the CD154 MicroBead Kit.
- Isolation of viable NY-ESO-1-specific CD4<sup>+</sup> and CD8<sup>+</sup> 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 NY-ESO-1-specific CD4<sup>+</sup> and CD8<sup>+</sup> 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.

## 2. Recommendations for *in vitro* restimulation of NY-ESO-1-specific T cells with PepTivator® NY-ESO-1 – premium grade

### 2.1 Cell preparation

For induction of cytokine secretion by NY-ESO-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](http://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](http://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® NY-ESO-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® NY-ESO-1 – premium grade fill a sterile syringe (0.5 mL) with 200 µL of sterile water. To dissolve the 60 nmol PepTivator NY-ESO-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 NY-ESO-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 NY-ESO-1-specific T cells

▲ NY-ESO-1-specific T cells are expected to be present only in certain individuals. Their frequency may be very low compared to T cells with other specificities. The given protocol for *in vitro* T cell stimulation thus may only serve as a guideline and is based on experiences using other PepTivator products, for example, PepTivator CMV pp65 – premium grade.

▲ 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<sup>7</sup> cells/mL. Plate cells in dishes at a density of 5×10<sup>6</sup> cells/cm<sup>2</sup> (refer to 4. Appendix: Flask and dish sizes for *in vitro* T cell stimulation).
3. Mix the reconstituted PepTivator NY-ESO-1 – premium grade thoroughly. Add 20 µL of PepTivator NY-ESO-1 – premium grade stock solution per mL cell suspension. Mix carefully and incubate cells at 37 °C; 5–7% CO<sub>2</sub>.

The final concentration of PepTivator NY-ESO-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. Jäger, E. *et al.* (2000) Monitoring CD8 T cell responses to NY-ESO-1: Correlation of humoral and cellular immune responses. *Proc. Natl. Acad. Sci. USA* 97: 4760–4765.

#### 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  $5 \times 10^6$  cells/cm<sup>2</sup>. 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
$0.15 \times 10^7$	0.15 mL	96 well	0.64 cm
$0.50 \times 10^7$	0.50 mL	48 well	1.13 cm
$1.00 \times 10^7$	1.00 mL	24 well	1.60 cm
$2.00 \times 10^7$	2.00 mL	12 well	2.26 cm
$5.00 \times 10^7$	5.00 mL	6 well	3.50 cm
Total cell number	Medium volume to add	Culture dish	Dish diameter
$4.5 \times 10^7$	4.5 mL	small	3.5 cm
$10.0 \times 10^7$	10.0 mL	medium	6 cm
$25.0 \times 10^7$	25.0 mL	large	10 cm
$50.0 \times 10^7$	50.0 mL	extra large	15 cm
Total cell number	Medium volume to add	Culture flask	Growth area
$12 \times 10^7$	12 mL	50 mL	25 cm <sup>2</sup>
$40 \times 10^7$	40 mL	250 mL	75 cm <sup>2</sup>
$80 \times 10^7$	80 mL	720 mL	162 cm <sup>2</sup>
$120 \times 10^7$	120 mL	900 mL	225 cm <sup>2</sup>

Refer to [www.miltenyibiotec.com](http://www.miltenyibiotec.com) for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit [www.miltenyibiotec.com/local](http://www.miltenyibiotec.com/local) to find your nearest Miltenyi Biotec contact.

#### Legal notices

##### Limited product warranty

Miltenyi Biotec B.V. & Co. KG and/or its affiliate(s) warrant this product to be free from material defects in workmanship and materials and to conform substantially with Miltenyi Biotec's published specifications for the product at the time of order, under normal use and conditions in accordance with its applicable documentation, for a period beginning on the date of delivery of the product by Miltenyi Biotec or its authorized distributor and ending on the expiration date of the product's applicable shelf life stated on the product label, packaging or documentation (as applicable) or, in the absence thereof, ONE (1) YEAR from date of delivery ("Product Warranty"). Miltenyi Biotec's Product Warranty is provided subject to the warranty terms as set forth in Miltenyi Biotec's General Terms and Conditions for the Sale of Products and Services available on Miltenyi Biotec's website at [www.miltenyibiotec.com](http://www.miltenyibiotec.com), as in effect at the time of order ("Product Warranty"). Additional terms may apply. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF A PRODUCT IS SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION METHODS.

##### Technical information

The technical information, data, protocols, and other statements provided by Miltenyi Biotec in this document are based on information, tests, or experience which Miltenyi Biotec believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such technical information and data are intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information. Miltenyi Biotec shall not be liable for any technical or editorial errors or omissions contained herein.

All information and specifications are subject to change without prior notice. Please contact Miltenyi Biotec Technical Support or visit [www.miltenyibiotec.com](http://www.miltenyibiotec.com) for the most up-to-date information on Miltenyi Biotec products.

##### Licenses

This product and/or its use may be covered by one or more pending or issued patents and/or may have certain limitations. Certain uses may be excluded by separate terms and conditions. Please contact your local Miltenyi Biotec representative or visit Miltenyi Biotec's website at [www.miltenyibiotec.com](http://www.miltenyibiotec.com) for more information.

The purchase of this product conveys to the customer the non-transferable right to use the purchased amount of the product in research conducted by the customer (whether the customer is an academic or for-profit entity). This product may not be further sold. Additional terms and conditions (including the terms of a Limited Use Label License) may apply.

CUSTOMER'S USE OF THIS PRODUCT MAY REQUIRE ADDITIONAL LICENSES DEPENDING ON THE SPECIFIC APPLICATION. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING FOR ITSELF WHETHER IT HAS ALL APPROPRIATE LICENSES IN PLACE. Miltenyi Biotec provides no warranty that customer's use of this product does not and will not infringe intellectual property rights owned by a third party. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

##### Trademarks

MACS, the Miltenyi Biotec logo, and PepTivator are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide.

Copyright © 2020 Miltenyi Biotec and/or its affiliates. All rights reserved.