

Contents

1. Description
 - 1.1 Background information
 - 1.2 Applications
2. Recommendations for *in vitro* restimulation of antigen-specific T cells with PepTivator® Peptide Pools
 - 2.1 Cell preparation
 - 2.2 Reagent requirements
 - 2.3 Recommendations for reconstitution of PepTivator® Peptide Pools
 - 2.4 Recommendation for *in vitro* restimulation of antigen-specific T cells
3. References
4. Appendix: Flask and dish sizes for *in vitro* T cell stimulation

1. Description

This product is for research use only.

Components	<p>6 nmol/peptide PepTivator® SARS-CoV-2 Prot_S1 – research grade</p> <p>or</p> <p>60 nmol/peptide PepTivator® SARS-CoV-2 Prot_S1 – research grade:</p> <p>Pool of lyophilized peptides, consisting mainly of 15-mer sequences with 11 amino acids (aa) overlap, covering the N-terminal S1 domain of the surface glycoprotein (“S”) of SARS-Coronavirus 2 (GenBank MN908947.3, Protein QHD43416.1). The PepTivator® SARS-CoV-2 Prot_S1 contains the aa sequence 1–692 of the surface glycoprotein, also termed “spike” protein.</p>
Capacity	<p>6 nmol (approximately 10 µg) per peptide for stimulation of up to 10⁸ total cells or 60 nmol (approximately 100 µg) per peptide for stimulation of up to 10⁹ total cells.</p>
Product format	Lyophilized peptides containing stabilizer.
Purity	Average purity of peptides >70% (HPLC).
Storage	Store lyophilized product at –20 °C. The expiration date is indicated on the vial label.

This product contains no preservative; always handle under aseptic conditions.

1.1 Background information

SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), also known as novel coronavirus 2019-nCoV, causes fever, severe respiratory illness, and can lead to life threatening pneumonia. The first cases of this disease, termed COVID-19 for coronavirus disease 2019, have been detected in December 2019 in Wuhan, China. SARS-CoV-2 Prot_S1 stands for the S1 domain of the surface glycoprotein of SARS CoV-2, the “spike protein”.

This protein is responsible for the recognition and binding of the coronavirus to the host cell. Once SARS-CoV-2 has bound to the ACE2 receptor of the host cell, fusion of viral envelope and host cell membrane starts, which enables the viral genome to enter the host cell. The spike protein consists of two functional domains: The S1 domain contains the surface binding site to the ACE2 receptor and the S2 subunit mediates membrane fusion. Cleavage at the S1/S2 boundary (aa-residues 685 and 686) via cellular proteases is crucial to prime the protein for the entry to the host cell.¹ The cleavage site distinguishes SARS-CoV-2 from SARS-CoV spike protein sequence, and is thought to be an important feature not only for its zoonotic potential^{2,3} but also for increased SARS-CoV-2 transmissibility^{1,4}. Thus, the spike proteins, and especially the S1 domain, are crucial for the infection of cells with coronaviruses and have been suggested as possible target for vaccine development.

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.
- Isolation of viable antigen-specific CD4⁺ T cells with the CD154 MicroBead Kit.
- 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 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.

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) Cytokine Secretion Assay Kit.
- (Optional) Antibodies or kits for intracellular cytokine staining, e.g., Anti-IFN-γ-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).
- (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® 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. Instead of water 20% DMSO can be used.
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

▲ **Coronavirus-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 marker, 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. 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. Plate cells in dishes at a density of 5×10⁶ cells/cm² (refer to 4. 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% CO₂.

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 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. References

1. Hoffmann, M. *et al.* (2020) SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181(2): 271–280.
2. Yang, Y. *et al.* (2014) Receptor usage and cell entry of bat coronavirus HKU4 provide insight into bat-to-human transmission of MERS coronavirus. *Proc. Natl. Acad. Sci. USA* 111(34): 12516–12521.
3. Menachery, V. D. *et al.* (2020) Trypsin treatment unlocks barrier for zoonotic bat coronavirus infection. *J. Virol.* 94(5): pii: e01774–19.
4. Li, W. *et al.* (2017) Identification of sialic acid-binding function for the Middle East respiratory syndrome coronavirus spike glycoprotein. *Proc. Natl. Acad. Sci. USA* 114 (40): E8508–E8517.

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 cm ²
40×10^7	40 mL	250 mL	75 cm ²
80×10^7	80 mL	720 mL	162 cm ²
120×10^7	120 mL	900 mL	225 cm ²

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.

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, 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 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 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, PepTivator, and TexMACS 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.