Miltenyi Biotec

PepTivator® AdV5 Hexon – premium grade

human

6 nmol/peptide	130-093-495
60 nmol/peptide	130-093-496

Contents

1. Description
   - 1.1 Background information
   - 1.2 Applications
2. Recommendations for *in vitro* restimulation of antigen-specific T cells with PepTivator® AdV5 Hexon – premium grade
   - 2.1 Cell preparation
   - 2.2 Reagent requirements
   - 2.3 Recommendations for reconstitution of PepTivator® AdV5 Hexon – premium grade
   - 2.4 *In vitro* restimulation of antigen-specific T cells
3. Examples
   - 3.1 Detection and isolation of viable AdV5 Hexon–specific T cells using MACS® IFN-γ Secretion Assay – Cell Enrichment and Detection Kit (PE)
   - 3.2 Detection of AdV5 Hexon–specific T cells by intracellular staining with Anti-IFN-γ-PE
4. References
5. Appendix: Flask and dish sizes for *in vitro* T cell stimulation

1. Description

Components	**6 nmol/peptide PepTivator® AdV5 Hexon – premium grade** or **60 nmol/peptide PepTivator AdV5 Hexon – premium grade:** Pool of lyophilized peptides, consisting mainly of 15-mer sequences with 11 amino acids (aa) overlap, covering the complete sequence of the hexon protein of human adenovirus 5 (AdV-5) (Swiss-Prot Acc. no. P04133).
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 preservative and is sterile filtered; always handle under aseptic conditions.

1.1 Background information

Adenoviruses are non-enveloped, ubiquitous, lytic, double-stranded DNA viruses. Up to now 52 different human serotypes (1–52), divided into six species (A–F) are described. Primary infections are usually early in life – either asymptomatic or with mild respiratory or gastrointestinal symptoms. Afterwards the viruses establish latency with persistent infection. Severe and life-threatening acute infections are frequently observed in hematopoietic stem cell transplantations, especially in children, and in solid organ transplantations.
The hexon protein is one of the structural proteins in the viral coat and is synthesized during late infection. It is the immunodominant T cell target among capsid proteins and contains multiple epitopes conserved among serotypes[1–8].

The PepTivator® AdV5 Hexon – premium grade is specially developed for efficient *in vitro* stimulation of hexon-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[9]. Stimulation of T cells with PepTivator AdV5 Hexon AdV5 Hexon – premium grade causes the secretion of effector cytokines and upregulation of activation markers, which then allows for detection or isolation of AdV5 hexon–specific T cells. Quantitative, phenotypical, or functional analysis of AdV5 hexon–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 AdV5 hexon–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 AdV5 hexon–specific $CD4^+$ T cells with the CD154 MicroBead Kit.
- Isolation of viable AdV5 hexon–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 immunotherapy in AdV infection.
- Generation of AdV5 hexon–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.

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

140-002-310.05

2. Recommendations for *in vitro* restimulation of antigen–specific T cells with PepTivator® AdV5 Hexon – premium grade

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.

2.2 Reagent requirements

- Culture medium, e.g., RPMI 1640 (# 130-091-440) containing 5% human serum, e.g., autologous or AB serum.
  ▲ **Note:** Do not use BSA or FCS because of non-specific stimulation.
- (Optional) Cytokine Secretion Assay Kit. For additional reagent and instrument requirements refer to the data sheet of the respective Cytokine Secretion Assay.
- (Optional) Intracellular cytokine staining, e.g., with Anti-IFN-γ-PE (# 130-091-653). For additional reagent requirements refer to the respective data sheet. For more information on other fluorochrome-conjugates see www.miltenyibiotec.com.
- (Optional) Intracellular cytokine staining of activated $CD4^+$ T cells by using, for example, the CD154/IFN-γ/CD4 Detection Kit (# 130-092-814).
- (Optional) CD154 MicroBead Kit (# 130-092-658). For details see the CD154 MicroBead Kit data sheet.
- (Optional) CD137 MicroBead Kit (# 130-093-476). For details see the CD137 MicroBead Kit data sheet.
- (Optional) CytoStim for restimulation of human T cells (# 130-092-172, # 130-092-173). For details see the CytoStim data sheet.
- (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® AdV5 Hexon – 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 AdV5 Hexon – premium grade fill a sterile syringe (0.5 mL) with 200 µL of sterile water. To dissolve the 60 nmol PepTivator – AdV5 Hexon – 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 AdV5 Hexon – 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 *In vitro* restimulation of antigen–specific T cells

▲ Always include a negative control (without antigen) in the experiment. A positive control (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 10^7 cells/mL. Plate cells in dishes at a density of 5×10^6 cells/cm² (see 5. Appendix: Flask and dish sizes for *in vitro* T cell stimulation).
3. Mix the reconstituted PepTivator Peptide Pool thoroughly. Add 20 µL of peptide pool stock solution per mL cell suspension. Mix carefully and incubate cells at 37 °C; 5% CO_2. The final concentration of 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.

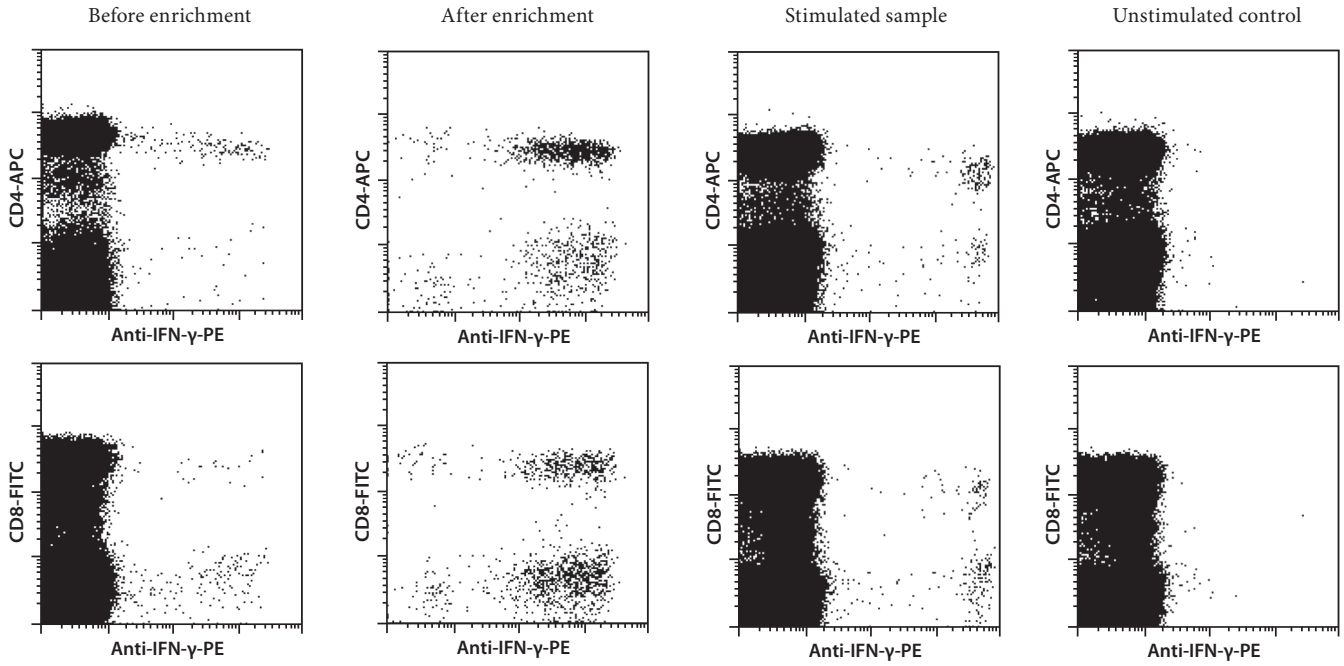
3. Examples

3.1 Detection and isolation of viable AdV5 Hexon–specific T cells using MACS® IFN-γ Secretion Assay – Cell Enrichment and Detection Kit (PE)

From an AdV$^+$ donor 10^7 human PBMCs were restimulated for 4 hours with 20 µL/mL of reconstituted PepTivator AdV5 Hexon – premium grade. AdV5 Hexon–specific cells were stained and magnetically enriched according to their secretion of IFN-γ using the IFN-γ Secretion Assay – Cell Enrichment and Detection Kit (PE) (# 130-054-201). T cells were counterstained for CD4 and CD8 expression. Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence. IFN-γ secretion of viable lymphocytes is shown.

3.2 Detection of AdV5 Hexon–specific T cells by intracellular staining with Anti-IFN-γ-PE

From an AdV$^+$ donor 10^6 human PBMCs were restimulated for 6 hours with 20 µL/mL of reconstituted PepTivator AdV5 Hexon – premium grade or without antigen. After 2 hours 1 µg/mL Brefeldin A was added. Cells were fixed, permeabilized, and AdV5 Hexon–specific cells were intracellularly stained with Anti-IFN-γ-PE (# 130-091-653). T cells were counterstained for CD4 and CD8 expression. IFN-γ production of lymphocytes is shown.



4. References

1. Olive, M. *et al.* (2002) The adenovirus capsid protein hexon contains a highly conserved human CD4$^+$ T cell epitope. Human Gene Therapy 13: 1167–1178.
2. Tang, J. *et al.* (2004) Adenovirus hexon T cell epitipe is recognized by most adults and is restricted by HLA DP4, the most common class II allele. Gene Therapy 11: 1406–1415.
3. Leen, A. *et al.* (2004) Conserved CTL epitopes on the adenovirus hexon protein expand subgroup cross-reactive and subgroup-specific CD8$^+$ T cells. Blood 104: 2432–2440.
4. Tang, J. *et al.* (2006) Human CD8$^+$ cytotoxic T cell responses to adenovirus capsid proteins. Virology 350: 312–322.
5. Veltrop-Duits, L. *et al.* (2006) Human CD4$^+$ T cells stimulated by conserved adenovirus 5 hexon peptides recognize cells infected with different species of human adenovirus. Eur. J. Immunol. 36: 2410–2423.
6. Haveman, L. *et al.* (2006) Novel pan-DR-binding T cell epitopes of adenovirus induce pro-inflammatory cytokines and chemokines in healthy donors. International Immunology 18: 1521–1529.
7. Onion, D. *et al.* (2007) The CD4$^+$ T cell response to adenovirus is focused against conserved residues within the hexon protein. J. Gen. Virol. 88: 2417–2425.
8. Leen, A. M. *et al.* (2008) Identification of hexon-specific CD4 and CD8 T-cell epitopes for vaccine and immunotherapy. J. Virol. 82: 546–554.
9. Kiecker, F. *et al.* (2004) Analysis of antigen-specific T-cell responses with synthetic peptides — what kind of peptide for which purpose? Hum. Immunol. 65: 523–536.

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

For *in vitro* T cell stimulation (see 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/cm^2. 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, and PepTivator are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide.