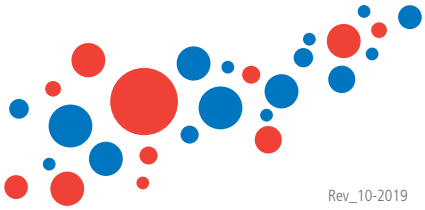
C.T.L.

Product Data Sheet



Rev_10-2019

EBV-MHC Class I Control Peptide Pool

Catalog #: CTL-EBV-001 or PA-EBV-001

Lot: _____

Product: EBV-MHC Class I Control Peptide Pool

Formulation: Each vial contains a total of 300µg lyophilized peptide powder consisting of 15 individual peptides at 20µg peptides each. **Remark:** The filling height in between vials of the same lot varies due to the lyophillization process. This does neither alter the functionality of the product, nor the amounts of peptides different.

Lot specific QC: Available on request

Description: The EBV-MHC Class I Control Peptide Pool contains 15 peptides, each corresponding to a defined HLA class I-restricted T cell epitope from Epstein Barr virus.

Activity: The EBV-MHC Class I Control Peptide Pool stimulates corresponding peptide-specific CD8+ memory T cells to release IFN-γ. These peptides are restricted by HLA- A2, -A3, -A11, -A24, -B7, -B8, -B27, -B44 molecules and have been shown to elicit recall responses in the majority of individuals expressing these rather common MHC class I alleles – most humans have been previously exposed to these pathogens. Because of promiscuous binding motifs, these peptides can occasionally bind other MHC class I, but not class II molecules, further extending the range of individuals in whom they elicit a CD8 cell recall response. Therefore, the majority of randomly selected human donors will respond to the EBV-MHC Class I Control Peptide Pool.

Recommended Use: The EBV-Control Peptide Pool is recommended as the positive control for detecting antigen-specific CD8+ cells in human PBMC, for example, when performing cytokine assays for immune monitoring purposes. Such assays include IFN-γ measurements by ELISPOT and intracytoplasmic cytokine staining (ICS), for accurate frequency measurements of the cytokine producing CD8 cells, or cytokine bead arrays (CBA), and ELISA, for a semi-quantitative read out.

Instruction for Use: **Stock solution:** Flick tube to ensure all powder is at the bottom of the tube. Add 10µl tissue culture grade DMSO followed by 40µl of sterile double distilled water. Vortex briefly and watch sterile handling! Add 450µl of tissue culture grade PBS and vortex briefly. Flick tube to ensure that all liquid is at the bottom of the tube. The stock solution is ready for use. This stock solution (500µl, 20x) contains each peptide at 40µg/ml concentration. The stock can be stored at 4°C for one week. CTL recommends to aliquot and store the stock solution at -20°C to -80°C for long-term storage.

Use peptide pool at 2µg/ml of final peptide concentration, that is at 1:20 dilution of the stock. For example, prepare a 1:10 dilution of the stock in tissue culture medium as the working solution, and add it 1+1 (v/v) to the medium containing the PBMC. For ELISPOT assays we recommend plating 100µl of this 2x peptide working solution per well, directly into the ELISPOT plate, followed by

adding the 100µl of the PBMC for a 24h assay duration. For exact frequency measurements, we recommend testing in triplicate. Because serum is the greatest variable in assay performance, we recommend the use of serum-free media at all steps of the assay. CTL offers such serum-free media (CTLT-005) that has been customized for low background/high signal performance with PBMC.

Storage: In lyophilized powder form store refrigerated at 4°C. The stock solution can be stored at 4°C for one week; however, aliquoted and stored frozen at -20° to -80˚C is more suitable for long-term storage.

Peptides: CLGGLLTMV (EBV, HLA-A2), GLCTLVAML (EBV, HLA-A2), RVRAYTYSK (EBV, HLA-A3), RLRAEAQVK (EBV, HLA-A3), AVFDRKSDAK (EBV, HLA-A11), IVTDFSVIK (EBV, HLA-A11), ATIGTAMYK (EBV, HLA-A11), DYCNVLNKEF (EBV, HLA-A24), RPPIFIRRL (EBV, HLA-B7), RAKFKQLL (EBV, HLA-B8), FLRGRAYGL (EBV, HLA-B8), QAKWRLQTL (EBV, HLA-B8), RRIYDLIEL (EBV, HLA-B27), YPLHEQHGM (EBV, HLA-B35), EENLLDFVRF (EBV, HLA-B44),

References:
Currier JR, Kuta EG, Turk E, Earhart LB, Loomis-Price L, Janetzki S, Ferrari G, Birx DL, Cox JH. A panel of MHC class I restricted viral peptides for use as a quality control for vaccine trial ELISPOT assays. *J. Immunol. Methods,* 260:157-172, 2002.

Mwau M. Michael A and Hanke T. Design and validation of an enzyme-linked immunospot assay for use in clinical trials of candidate HIV vaccines. *AIDS Res. Hum. Retroviruses,* 18: 611-8, 2002.

For laboratory research use only. Not for use in diagnostic or therapeutic procedures.

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