

Immune Monitoring

FRED HUTCHINSON CANCER RESEARCH CENTER SEATTLE, WA • 501(C)(3) NONPROFIT



Shared Resource: Immune Monitoring Location: Steam Plant Building, S5-101 Contact phone: (206) 667-6455 Contact fax: (206) 667-6845 Contact email: jcao@fredhutch.org

MHC Class-I Tetramers

pMHC tetramers are multimeric peptide-MHC complexes that serve as reagents for analyzing antigen-specific T cells. The multimeric nature of tetramers increases the affinity between pMHC and TCR enabling their usage as labeling reagents for TCR. Labeling of antigen-specific T cells by tetramers makes possible the characterization of the phenotype and functionality of these cell populations.

In the production of tetramers, the desired MHC heavy chain protein and epitopic peptide are refolded into MHC complex with β 2microglobulin in vitro. The successful refolded monomers are purified by gel-filtration and then biotinylated. Tetramerization is achieved by binding to streptavidin, a fluorescent dye often added by using fluorochrome-conjugated streptavidin.

Our core produces pMHC monomers and tetramers with defined MHC alleles and epitopic peptides. See the table below for available MHC Class-I alleles. We offer tetramers conjugated with, but not limited to, PE, APC, BV421, BV711, BB515 and AlexaFluor 647. Contact us for more details.

Note: Individual tetramers can differ significantly in refolding, stability and staining of T cells. The differences are determined largely by the HLA alleles and the binding affinity of the specific peptide to the HLA molecule. We recommend the testing of each new batch of tetramers with positive and negative control cells and by titration of the tetramer reagent. Tetramers should be stored at 4°C and retested after long-term storage. Please visit the <u>NIH Tetramer Core Facility's website</u> for more information.

High-Grade Tetramers

We produce high-grade pMHC Class-I tetramers. These custom-made, sterile and highly purified products are used to sort antigen-specific CD8+ T cells, which are cultured and then used for immunotherapy trials. The production of these tetramers is approved by the FDA to use in certain IND protocols at Fred Hutchinson Cancer Research Center.

Table 1. Available MHC Class-I alleles for tetramer production

| Human | | | | |
|---|--|---|--|----------------------------------|
| A*0101 A*0201 A*0201 M (A245V) A*0201 DM (D227K/T228A) A*0301 | B*0702 B*0801 B*1402 B*1501 B*1801 | Cw*0101 Cw*0202 Cw*0701 Cw*0702 Cw*1203 | HLA-E0101 HLA-E0103 HLA-F HLA-G | H-2Db H-2Kb H-2Kd H-2Dd |

| A*1101 | B*3501 | |
|---------|--------|--|
| A*2301 | B*3502 | |
| A*24021 | B*4001 | |
| A*2501 | B*4002 | |
| A*2902 | B*5101 | |
| A*3002 | B*5201 | |
| A*3101 | | |
| A*68012 | | |

Requesting Service

Please use <u>iLab</u> to request any of the above assays.