Competition Assay Technology

Competition based peptide binding assay methodologies have become exceedingly popular for assessing the ability of synthetically defined peptide epitopes to associate with specific HLA complexes. This has been accomplished by determining their half maximal inhibitory concentration (IC50) as a measure of the effectiveness of an inhibiting test peptide to bind its target molecule.

We help researchers to design improved vaccination and therapeutic targeting strategies, by quickly advancing qualified candidates through the development process by facilitating peptide affinity confirmation and ranking based on real binding characteristics using our novel, fluorescence polarization (FP) based competition assay procedure.

FP Technology

Fluorescence polarization (FP) is unique among methods used to analyze molecular binding events because it allows the instantaneous measurement of the ratio between a free and bound labeled ligand in solution without any separation steps. The technology is based on the principle that if a fluorescent-labeled peptide binds to the sHLA molecule of higher molecular weight, polarization values will increase due to the slower molecular rotation of the bound probe.

sHLA Technology

Pure Protein, L.L.C. is the world leader in HLA production from mammalian cell lines. All proteins are single specificity, naturally loaded, and processed through the cell. After years of development, Pure Protein is excited to offer best in class reagents and services to customers at www.HLAProtein.com.

Contact Us

FOR GENERAL INQUIRIES

(844) 444-sHLA or (844) 444-7452

info@hlaprotein.com

FOR TECHNICAL SUPPORT

support@hlaprotein.com

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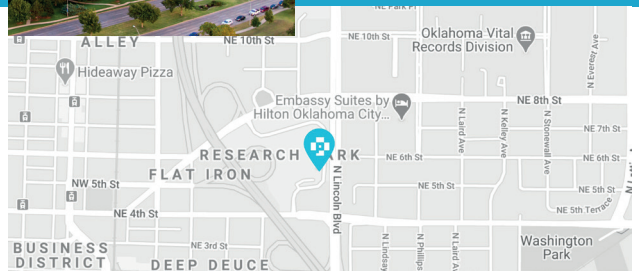
orders@hlaprotein.com

FOR COMMERCIAL LICENSING

licensing@hlaprotein.com

RESEARCH FACILITIES

655 Research Parkway
Suite 556
Oklahoma City, OK 73104



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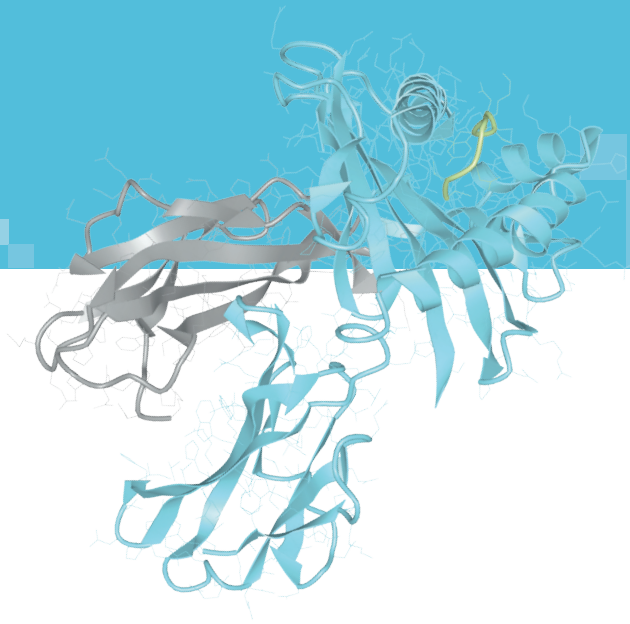
HLA Protein

PURE PROTEIN LLC



Peptide Epitope Validation

HLA Class I

Competition-Based
Peptide Binding Assays

Accurately determine IC_{50} Values

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Validation

How it Works

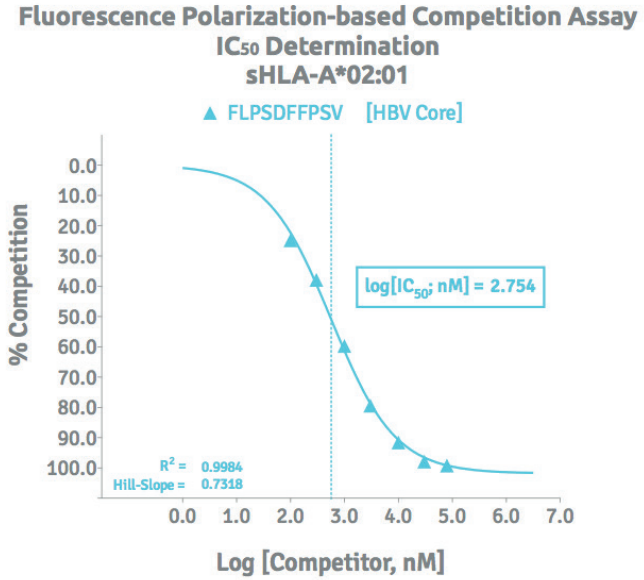
Provide a synthetic peptide
Select peptides from screening results derived from target source proteins in cancer and infectious disease applications to putative epitopes originated from predictive algorithms, altered or mutated peptides from viral variance studies, or tumor-specific mutations.

Select an HLA molecule
Select from a broad representation of HLA alleles to be applied in our peptide binding assays. Our service is very flexible, ensures expedited results, and employs highly-purified single specificity soluble HLA proteins from mammalian cell lines. We guarantee outstanding performance in accuracy, sensitivity, and reproducibility.

Receive the data
Inhibitory concentrations are determined by incubating sHLA with a labeled reference peptide in the presence of different concentrations of a competitor peptide. We deliver a calculated logIC50 value as measure of the effectiveness of the competing test peptide. Affinity categories will prioritize your logIC50 values into high, medium, or low affinity binders.

Peptide Binding Assay Results
A logIC50 value is determined by plotting the response values as a function of the logarithms of competitor concentrations and applying a nonlinear regression dose response model with variable slope for analysis.

Sample Data



Affinity Categories					
High Affinity	Medium Affinity	Low Affinity	Very Low Affinity	No Binder	
<	3.700	4.700	5.500	6.000	log(IC_{50}; nM)
3.700	4.700	5.500	6.000	>	
<	5,000	50,000	350,000	1,000,000	IC_{50} (nM)
5,000	50,000	350,000	1,000,000	>	

Available Assays

PP-A*01:01	PP-B*07:02	PP-B*35:08
PP-A*02:01	PP-B*07:03	PP-B*40:02
PP-A*02:02	PP-B*08:01	PP-B*42:01
PP-A*02:03	PP-B*15:01	PP-B*44:02
PP-A*02:05	PP-B*15:02	PP-B*53:01
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PP-A*29:02	PP-B*27:05	PP-B*67:01
PP-A*34:02	PP-B*27:08	PP-B*81:01
PP-A*36:01	PP-B*35:01	

Validation Options

- Validate Screening Hits
- Validate Mutational Variations
- Validate Predicted Peptides
- Validate Peptide Modifications

Advantages

- Highly Accurate
- Rapid & Comprehensive
- Informative
- Flexible
- State-of-the-Art Detection System
- Unique MHC Class I Molecules
- Outstanding Performance
- No Radioisotopes
- Homogeneous Assays

Competition Assay Screening Technology

Competition based peptide binding assay methodologies have become exceedingly popular for assessing the ability of synthetically defined peptide epitopes to associate with specific HLA complexes. This has been accomplished by determining their half maximal inhibitory concentration (IC50) as a measure of the effectiveness of an inhibiting test peptide to bind its target molecule.

We help researchers to expedite their discovery process and significantly reduce the risks of their vaccine program by prioritizing epitopes with the greatest potential and highest population coverage based on real binding characteristics using our novel, fluorescence polarization (FP) based competition assay procedure.

FP Technology

Fluorescence polarization (FP) is unique among methods used to analyze molecular binding events because it allows the instantaneous measurement of the ratio between a free and bound labeled ligand in solution without any separation steps. The technology is based on the principle that if a fluorescent-labeled peptide binds to the sHLA molecule of higher molecular weight, polarization values will increase due to the slower molecular rotation of the bound probe.

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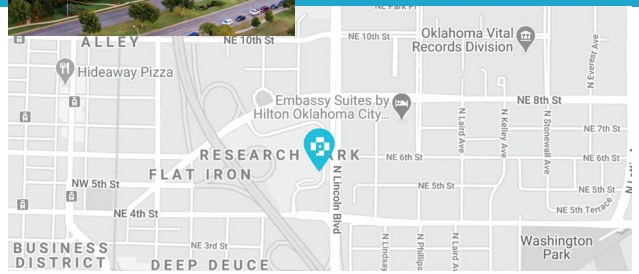

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HLA Protein

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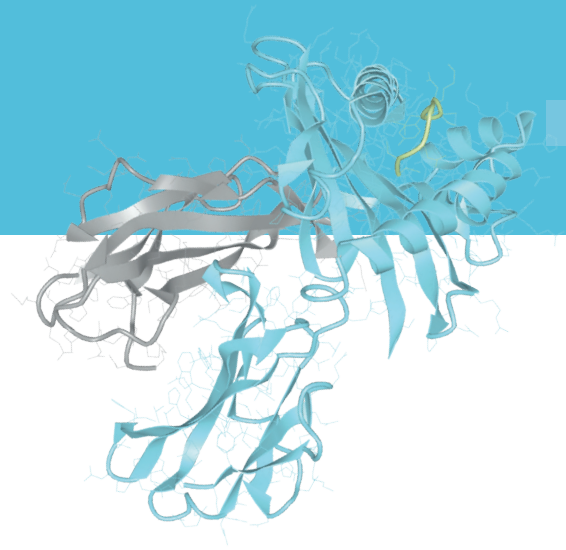

Peptide Epitope Screening

HLA Class I

High-Throughput Competition-Based Peptide Screening

Prioritize epitopes with the greatest potential

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Screening

How it Works

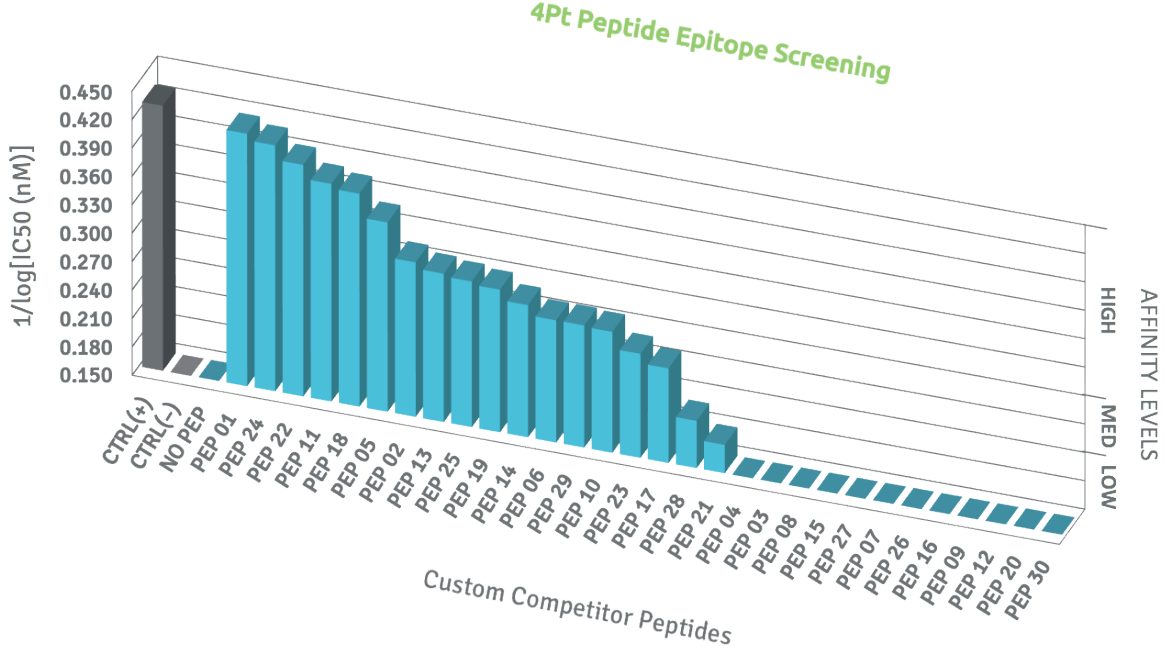
Select peptides and HLA allele(s)
Select from a broad panel of diverse libraries that fit your target(s) screening strategy with the intention of finding high-scoring peptide epitopes, successfully eliminating negative results, or optimizing your candidates among multiple alleles

Select Screening Mode
A 3 point screen is most often used by customers to scan large numbers of synthetic peptides with the end goal to obtain a competition score allowing a simple ranking of all candidates. The 3pt screen is primarily focused on the elimination of non-binding peptides. Peptides that exhibit binding above a predetermined threshold score are selected.

A 4 point screen generates a reduced dose-response curve that allows the approximation of logIC50 values based on a limited set of four experimental data points. It is often used by customers as a first pass evaluation to obtain more detailed data sets needed to better evaluate peptide effectiveness. Affinity categories will prioritize your logIC50 values into high, medium, or low affinity binders.

Receive Data Report
High affinity binding is the critical factor controlling immunogenicity of peptides. Results will be prioritized based on the highest ranked screening hits showing their HLA-type, screening score or approximate logIC50 value. Peptides with high vs low scores (logIC50) are easily identified and ranked in descending order.

Sample Data



Available Assays

PP-A*01:01	PP-B*07:02	PP-B*35:08
PP-A*02:01	PP-B*07:03	PP-B*40:02
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PP-A*34:02	PP-B*27:08	PP-B*81:01
PP-A*36:01	PP-B*35:01	

Screening Strategies

- Prediction Library
- Individual Library
- Mutational Library
- Focused Library
- Restriction Library
- Scanning Library

Advantages

- Ranks and Prioritizes Epitopes
- Wide Range of HLA Alleles
- High-Throughput Platform
- Customized Allele Selection
- Successful Elimination of Non-Binding Peptides
- Reliable Data Easy to Interpret
- Rapid, Comprehensive & Highly Reproducible
- Saves Valuable Resources and Costs
- Superior in Sensitivity and Specificity

Competition Assay Mapping Technology

Competition based peptide binding assay methodologies have become exceedingly popular for assessing the ability of synthetically defined peptide epitopes to associate with specific HLA complexes. This has been accomplished by determining their half maximal inhibitory concentration (IC50) as a measure of the effectiveness of an inhibiting test peptide to bind its target molecule.

We help researchers to significantly improve their peptide epitope discovery process, slash development costs and strengthen their intellectual property portfolio by accessing our most advanced combinatorial peptide library mapping approaches using our novel, fluorescence polarization (FP) based competition assay procedure.

FP Technology

Fluorescence polarization (FP) is unique among methods used to analyze molecular binding events because it allows the instantaneous measurement of the ratio between a free and bound labeled ligand in solution without any separation steps. The technology is based on the principle that if a fluorescent-labeled peptide binds to the sHLA molecule of higher molecular weight, polarization values will increase due to the slower molecular rotation of the bound probe.

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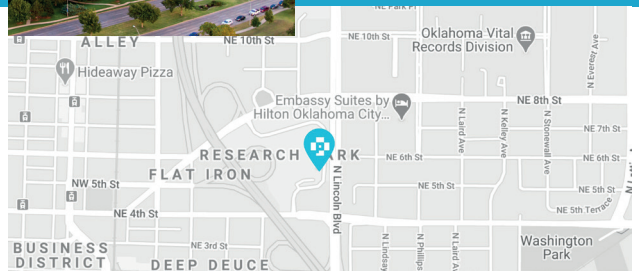

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HLA Protein

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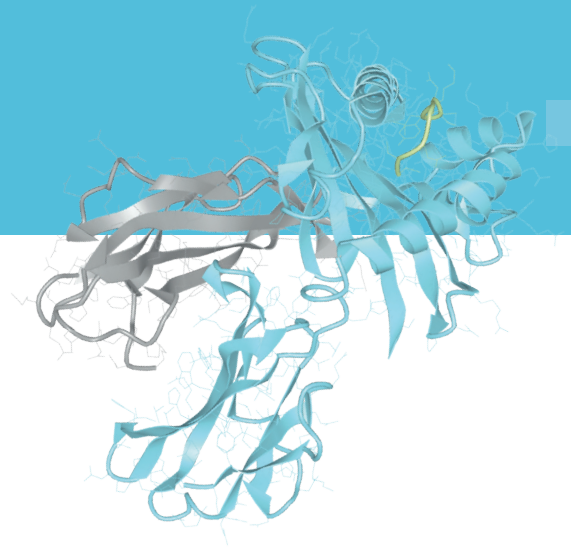

T cell Epitope Mapping

HLA Class I

High-Throughput Competition-Based Peptide Mapping

Identify novel epitope targets

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Mapping

How it Works

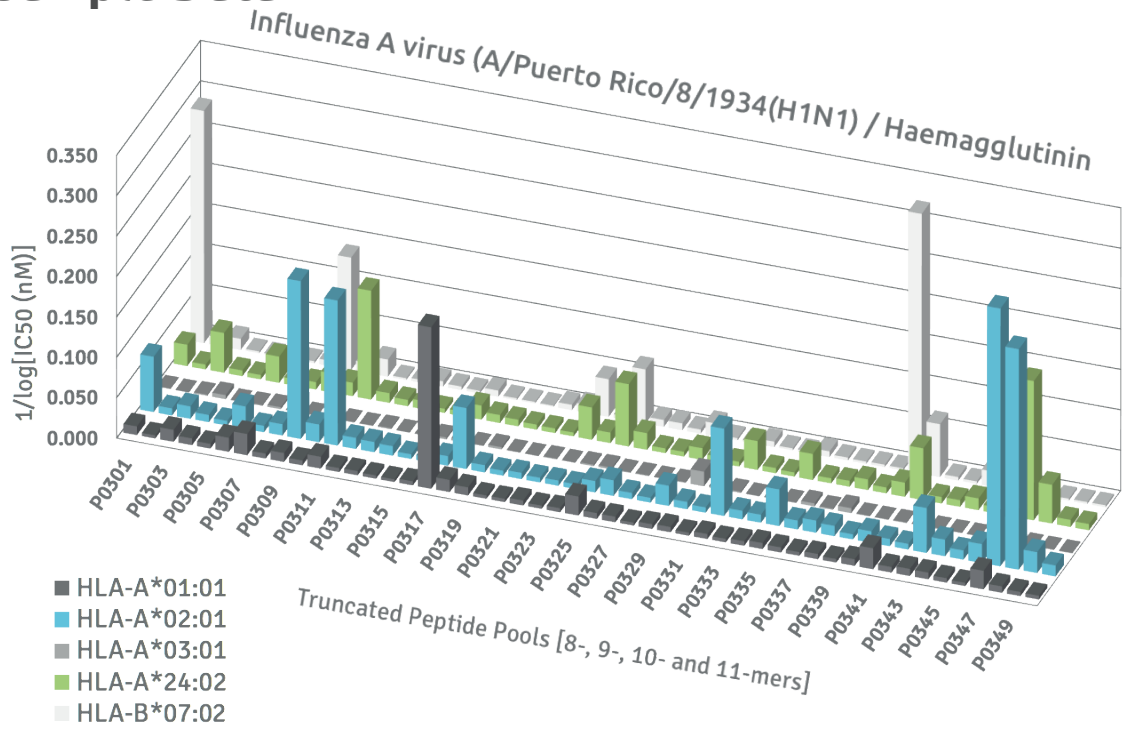
Select target protein, library modality and HLA Select a specific target from cancer antigens or pathogenic sequence that provides the source for your novel epitopes and choose a library modality to be specifically synthesized and screened with the HLA allele combination of your choice to identify the most potent T cell epitope candidates for your discovery program.

Receive High-Throughput Screening Hit Report
Receive primary screening results identifying candidates with affinities ranging from high to low using our state-of-the-art peptide screening assay. Moreover, the assay eliminates all non-binding peptides which need not be investigated further. The use of our technology in this primary screening will speed up your lead discovery process and deliver higher quality hits.

Prioritize Top Candidates
High affinity binding is the critical factor controlling immunogenicity of peptides. Within this refinement process, prioritize the highest ranked screening hits and validate their potential by generating dose–response curves determining their inhibitory concentration (IC_{50}) as a measure of their effectiveness.

Receive a Mapping Report
After completion of the screens, the first epitope map for a cancer or virus-related protein can be created, showing detailed information on the position of each epitope on the sequence string, their HLA-type, and screening score. In addition, the map uncovers the location of immunological hotspots and cross-reactivity patterns among all HLA-molecules investigated.

Sample Data



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PP-A*34:02	PP-B*27:08	PP-B*81:01
PP-A*36:01	PP-B*35:01	

Target Selection

Select from the wealth of newly available genomic sequence information that provides a superb source for the identification of novel peptide epitope targets derived from cancer antigens or other aberrant gene expressions. Targets may also be derived from viral, bacterial or other pathogenic origins, or from host proteins that are uniquely expressed, processed, modified or degraded.

Library Selection

“Never miss an epitope!” Select a truncation library of overlapping peptides with systematic truncation of the flanking residues allowing the screening of any possible combination of 8, 9, 10, and 11-mer of a chosen target protein for a seamless HLA Class I epitope discovery. Ensure that no epitope capable of producing an immune response is left behind.