



IEDB
Immune Epitope Database & Tools

T Cell Class II Tools

Binding, Processing, Immunogenicity

tools.iedb.org

Presented by: Dr. Bjoern Peters, Professor

CD4+ T cells Recognize MHC-II-bound Epitopes

- MHC-II molecules are constitutively expressed on professional antigen-presenting cells (APCs)
- APCs take up antigens from the extracellular environment (bacteria, secreted proteins, debris from dead cells, ...) and present them to CD4+ T cells
- CD4+ T cells provide 'help' to both CD8+ T cells and B cells, as well as having direct effector functions

MHC Class II Molecules

- Two MHC encoded polymorphic chains (α , β), which both impact the binding motif
- Both α and β chains are variable for DP & DQ loci
 - 'HLA-DPA1*01:03/DPB1*02:01'
 - 'HLA-DQA1*01:01/DQB1*05:01'
- Only β chain is variable for DR locus
 - HLA-DRB1*01:01
- DRB3/4/5 locus is in tight linkage with DRB1
 - Often not typed, but just as important

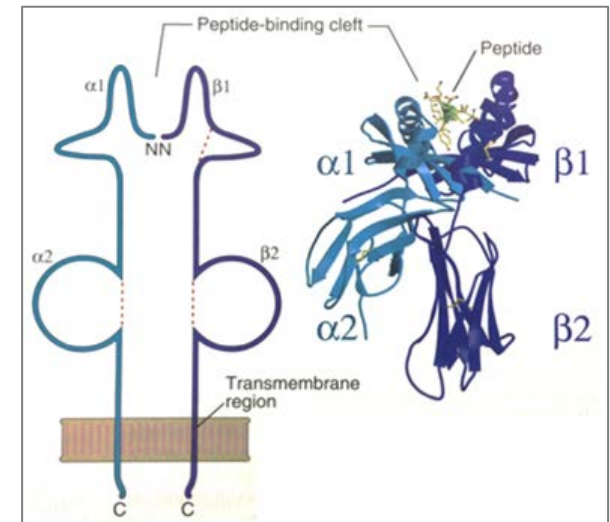
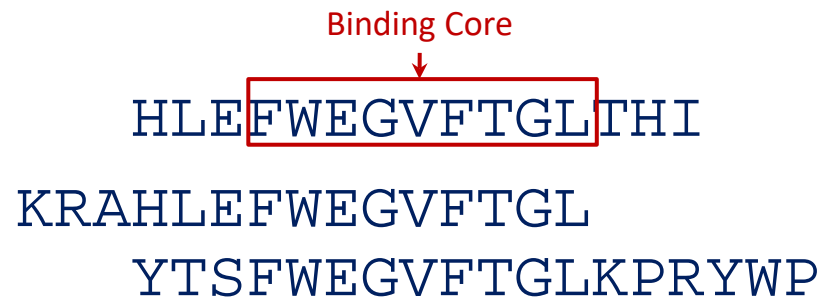


Figure Source
Cellular & Molecular Immunology, 5th Ed by Abbas and Lichtman

MHC Class II Peptide Binding

- Binding groove is open and can accommodate peptides (13-25 AA)
- 9 AA binding core within the peptide interacts with the binding groove of the MHC molecule



- Challenge: Correct identification of the binding core is not trivial; dissimilar-looking peptides can bind with identical affinity

T Cell – MHC Class II Binding Prediction

tools.iedb.org/main/tcell/

IEDB Analysis Resource

Overview | **T Cell Tools** | B Cell Tools | Analysis Tools | Tools-API | Usage | Download | Datasets | Contribute Tools | References

T Cell Epitope Prediction Tools

T Cell Epitopes - MHC Binding Prediction

These tools predict IC50 values for peptides binding to specific MHC molecules. Note that binding to MHC is necessary but not sufficient for recognition by T cells.

[Peptide binding to MHC class I molecules](#)

This tool will take in an amino acid sequence, or set of sequences and determine each subsequence's ability to bind to a specific MHC class I molecule.

[Peptide binding to MHC class II molecules](#)

This tool employs different methods to predict MHC Class II epitopes, including a consensus approach which combines NN-align, SMM-align and Combinatorial library methods.

[Tepitool](#)

The Tepitool provides prediction of peptides binding to MHC class I and class II molecules. Tool is designed as a wizard with 6 steps as described below. Each field (except sequences and alleles) is filled with default recommended settings for prediction and selection of optimum peptides. The input parameters can be adjusted as per your specific needs. You can go back to previous steps to change your selection before submission of the job. Once you submit the job (at the end of step-6), you will not be able to make any more changes and will have to start the prediction all over again with updated input parameters.

T Cell Epitopes - Processing Prediction

These tools predict epitope candidates based upon the processing of peptides in the cell.

[Proteasomal cleavage/TAP transport/MHC class I combined predictor](#)


This tool combines predictors of proteasomal processing, TAP transport, and MHC binding to produce an overall score for each peptide's intrinsic potential of being a T cell epitope.

[Neural network based prediction of proteasomal cleavage sites \(NetChop\) and T cell epitopes \(NetCTL and NetCTLpan\)](#)

NetChop is a predictor of proteasomal processing based upon a neural network. NetCTL and NetCTLpan are predictors of T cell epitopes along a protein sequence. It also employs a neural network architecture.

[MHC-NP: Prediction of peptides naturally processed by the MHC](#)

MHC-NP employs data obtained from MHC elution experiments in order to assess the probability that a given peptide is naturally processed and binds to a given MHC molecule. This tool was the winner of the [2nd Machine Learning Competition in Immunology](#).

 **MHCII-NP:**


This tool utilizes MHC II ligand elution data to predict naturally processed MHC II ligands by scanning the given peptide sequences.

T Cell Epitopes - Immunogenicity Prediction

This tool predicts the relative ability of a peptide/MHC complex to elicit an immune response.

[T cell class I pMHC immunogenicity predictor](#)

This tool uses amino acid properties as well as their position within the peptide to predict the immunogenicity of a class I peptide MHC (pMHC) complex.

 **Deimmunization:**

The deimmunization tool is attempt to identify immunodominant regions in a given therapeutically important protein, and suggest amino-acid substitutions that create non-immunogenic versions of the proteins. So we have opted a two steps process; 1) In the



MHC-II Binding Prediction Interface

- Tool entry point layout similar to class I

tools.iedb.org/mhcii/

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MHC-II Binding Predictions

Specify Sequence(s)

Enter protein sequence(s) in FASTA format

Or select file containing sequence(s) No file chosen

Choose a Prediction Method

Prediction Method [?](#) [Help on prediction method selections](#)

Show all the method versions:

Specify what to make binding predictions for

Select species/locus

Select MHC allele(s)
Select α & β chains separately if applicable: [?](#)

[Select full HLA reference set:](#) [?](#)

Select 7-allele HLA reference set: [?](#)

[?](#)

Select length(s) [?](#)

11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30

Specify Output

Sort peptides by

Output format

Email address (optional) [?](#)

MHC-II Binding Prediction Interface

- Tool entry point layout similar to class I

tools.iedb.org/mhcii/

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MHC-II Binding Predictions

Specify Sequence(s)

Enter protein sequence(s) in FASTA format

Or select file containing sequence(s) No file chosen

Choose a Prediction Method

Prediction Method [?](#)
Show all the method versions:

Select species/locus

Select MHC allele(s)
Select α & β chains separately if applicable: [?](#)

Select full HLA reference set: [?](#)
Select 7-allele HLA reference set: [?](#)

Select length(s) [?](#)

Specify Output

Sort peptides by: Percentile Rank

Output format: XHTML table

Email address (optional): [?](#)

Select prediction method

MHC II Binding Prediction Methods - Benchmarking

http://tools.iedb.org/auto_bench/mhcii/weekly/

MHC II Automated Server Benchmarks

This is a [live](#) ranking of MHC II servers based on performance, which continues to be reevaluated over time. The weekly IEDB releases are automatically checked for datasets large enough to add to the benchmarks. The benchmark metrics in the table below will only be updated on releases where such new data is becoming available.

Accumulated overall ranking scores

[Ranking scores](#) based on data sets submitted to the IEDB for the last at least 5 references.

Server	2023-09-01	2023-07-28	2023-04-21	2023-03-03	2023-01-27	2022-10-28	2022-09-16	2022-09-02	2022-06-17	2022-06-10	2022-04-08	2022-04-01	2021-12-10	2021-11-05	2021-10-29	2021-07-23	2021-04-23	2021-04-16	2021-02-19	2021-01-22	2020-12-18	2020-10-30	2020-10-23	2020-08-07	2020-06-26	2020-05-01	2020-03-27	2020-03-16	2020-01-03	2019-07-02	2019-05-24	2019-03-22	2018-11-23	
NetMHCIIpan-4.1 BA	85	82	70	71	70	70	76	65	65	65	65	65	67	66	65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NetMHCIIpan-4.0 BA	76	78	68	70	68	68	70	65	66	66	63	63	63	62	61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NetMHCIIpan-3.2	67	67	64	65	64	63	52	51	51	50	61	63	69	70	77	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NN-align 2.3	56	59	51	49	49	48	45	63	64	67	67	67	65	67	67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NetMHCIIpan-3.1	53	54	66	56	54	55	41	47	47	41	46	46	48	50	54	58	57	58	55	58	46	49	60	61	60	64	55	64	65	65	71	84	79	
Consensus IEDB method	50	58	58	59	59	61	57	66	66	68	65	66	63	62	63	66	66	71	73	69	73	76	62	60	60	58	58	61	64	66	64	64	64	64
NetMHCIIpan-4.1 EL	45	39	51	54	57	57	54	32	32	33	36	36	38	36	38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
STMM-align	43	42	50	50	47	48	42	55	56	52	50	47	50	51	53	49	51	43	43	43	57	61	57	57	54	56	38	41	43	39	43	49	35	
NN-align	38	42	44	43	43	42	43	58	58	57	57	59	55	56	57	68	64	73	73	75	59	60	73	74	78	79	88	75	71	71	66	54	63	
NetMHCIIpan-4.0 EL	27	24	47	47	50	51	48	31	31	29	34	33	37	34	38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tepitope (Sturmio)	26	30	39	39	41	41	37	31	27	41	36	37	41	39	29	21	24	17	18	14	21	29	27	25	27	26	33	28	26	26	29	21	47	
Comlib matrices	20	14	7	4	4	4	13	17	17	15	18	16	16	16	17	10	11	8	8	7	30	38	25	25	21	10	0	0	5	5	5	10	4	

Andreatta et al, Bioinformatics, 2017

- Similar to the MHC Class I benchmark set-up
- Binding affinity are best predicted with BA output; epitope selection is better using the EL output

MHC-II Binding Prediction – Example

tools.iedb.org/mhcii/

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MHC-II Binding Predictions

Specify Sequence(s)

Enter protein sequence(s) in FASTA format

```
>West Nile virus envelope glycoprotein
FNCLGMSNRDFLEGVSGATWVDLVLEGDSCVTIMSKDKPTIDVKMMMNMEANLAEVRSYCYLATVSDLST
KAACPMTMGEAHNDKRAPAFVCRQGVVDRGWNGCGLFGKGSIDTCAKFACTKAIGRTILKENIKYEVA
IFVHGPTTVESHGNYSTQVQAGTQAGRFSITPAAPSYTLKLGEGYEVTVDCPRSGIDTNAYYVMTVGTKT
FLVHREWFMDLNLWPSSAGSTVWRNRRETLMEFEPPHATKQSVIALGSQEGALHQAAGAIPEFSSNTVK
LTSGHLKCRVKMEKQLKGTTYGVCSKAFKFLGTPADTGHGTVVLELQYTGTDGPKVPPISSVASLNDLT
PVGRLVTVNPFSVATANAKVLIIELEPPFGDSYIVVGRGEQQINHHWHKSGSSIGKATFTTLKGAQRLLA
LGDTAWDFGVSQGGVFTSVGKAVHQVFGGAFRSLFGGMSWITQGLLGALLLWWMGINARDRSIALTFLAVG
GVLFLSVNVHA
```

Or select file containing sequence(s) No file chosen

Choose a Prediction Method

Prediction Method [?](#)
Show all the method versions:

NetMHCIIpan 4.1 EL (recommended epitope predictor-2023.09) [Help on prediction method selections](#)

Specify what to make binding predictions for

Select species/locus

Select MHC allele(s)
Select α & β chains separately if applicable: [?](#)

[Select full HLA reference set:](#) [?](#)
[Select 7-allele HLA reference set:](#) [?](#)

Select length(s) [?](#)

default 12-18 as is

11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30

Specify Output

Sort peptides by Percentile Rank

Output format XHTML table

Email address (optional) [?](#)

Choose species & locus

Allele Selection - α and β Chains

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MHC-II Binding Predictions

Specify Sequence(s)

Enter protein sequence(s) in FASTA format

Or select file containing sequence(s)

Prediction Method [?](#)
Show all the method versions:

Select species/locus

Select MHC allele(s)
Select α & β chains separately if applicable: [?](#)
[Select full HLA reference set:](#) [?](#)
Select 7-allele HLA reference set: [?](#)

Select length(s) [?](#)

Specify Output

Sort peptides by: Percentile Rank

Output format: XHTML table

Email address (optional) [?](#)

Method Selection: Extended epitope predictor-2023.09 [Help on prediction method selections](#)

Allele Selection: [Upload allele file](#) [?](#)

Length Selection: default 12-18 as is

11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30

Allele List (highlighted):

- DQA1*01:01/DQB1*02:01
- DQA1*01:01/DQB1*02:02
- DQA1*01:01/DQB1*02:03
- DQA1*01:01/DQB1*02:04
- DQA1*01:01/DQB1*02:05
- DQA1*01:01/DQB1*02:06
- DQA1*01:01/DQB1*03:01
- DQA1*01:01/DQB1*03:02
- DQA1*01:01/DQB1*03:03
- DQA1*01:01/DQB1*03:04
- DQA1*01:01/DQB1*03:05
- DQA1*01:01/DQB1*03:06
- DQA1*01:01/DQB1*03:07
- DQA1*01:01/DQB1*03:08
- DQA1*01:01/DQB1*03:09
- DQA1*01:01/DQB1*03:10
- DQA1*01:01/DQB1*03:11
- DQA1*01:01/DQB1*03:12
- DQA1*01:01/DQB1*03:13

Allele Selection - α and β Chains

tools.iedb.org/mhcii/

IEDB Analysis Resources

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MHC-II Binding Prediction

Enter protein sequence(s) in FASTA format

Or select file containing sequence(s)

Prediction Method [?]
Show all the method versions:

Select species/locus

Select MHC allele(s)
Select α & β chains separately if applicable:

Select full HLA reference set: [?]
Select 7-allele HLA reference set: [?]

Select length(s) [?]

DQA1*01:01/DQB1*02:01
DQA1*01:01/DQB1*02:02
DQA1*01:01/DQB1*02:03
DQA1*01:01/DQB1*02:04
DQA1*01:01/DQB1*02:05
DQA1*01:01/DQB1*02:06
DQA1*01:01/DQB1*03:01
DQA1*01:01/DQB1*03:02
DQA1*01:01/DQB1*03:03
DQA1*01:01/DQB1*03:04
DQA1*01:01/DQB1*03:05
DQA1*01:01/DQB1*03:06
DQA1*01:01/DQB1*03:07
DQA1*01:01/DQB1*03:08
DQA1*01:01/DQB1*03:09
DQA1*01:01/DQB1*03:10
DQA1*01:01/DQB1*03:11
DQA1*01:01/DQB1*03:12
DQA1*01:01/DQB1*03:13

VTIMSKDKPTIDVKMMNMEAANLAEVRSYC
SWGNGCGLFGKGSIDTCAKFACTKAIGRTI
WAPSYTLKLG EYGEVTV DCEPRSGIDTNAYY
IEFEEPHATKQSVIALGSQEGALHQALAGAI
LGT PADTGHGTVVLELQYTGTDGPCKVPIS

FASTA format detected.

prediction method selections

“7-Allele” Method

- Aim was to capture maximum immune response with minimum no. of peptides
- 6 peptide datasets with measured immune responses (SFCs/106 PBMCs)
- 15 or 16-mer peptide sets with 10 AA residues overlapping

Dataset	Purpose	No. of Antigens	Total peptides	No. of donors	Reference
Der p/f (House dust mite)	Training data	4	156	20	Hinz et al., 2015, CEA
Phl p (Timothy grass)	Training data	10	425	25	Oseroff et al., 2010, JI
TB-1	Training data	4	71	18	Arlehamn et al., 2012, JI
TB-2	Training data	11	499	32	Arlehamn et al., 2016, PLoS Path
Cockroach	Validation data	6	463	19	Dillon et al., 2015, CEA
Pertussis	Validation data	9	785	23	Bancroft et al., 2016, CEA
TOTAL		44	2399	137	

“7-Allele” Method

MHC-II Binding Predictions

Specify Sequence(s)

Enter protein sequence(s) in FASTA format

```
>West Nile virus envelope glycoprotein
FNCLGMSNRDFLEGVSGATWVDLVLEGDSCVTIMSKDKPTIDVKMMNMEAANLAEVRSYCYLATVSDL
STKAACPTMGEAHNDKRADPAFVCRQGVVDRGWNGCGLFGKGSIDTCAKACSTKAIGRTILKENIKY
EVAIFVHGPTTVESHGNYSTQVGATQAGRFSITPAAPSYTLKLGEGEVTVDCEPRSGIDTNAYYVMTVGT
KTLFVHREWFMDLNLWPSSAGSTVWRNRETLMEFEPPHATKQSVIALGSQEGALHQALAGAIPVEFSSN
TVKLTSGHLKCRVKMEKLQKGTTYGVCSKAFKFLGTPADTGHGTVVLELQYTGTDGPKVPISSVASLND
LTPVGRLVTVNPFVSVATANAKVLIELEPPFGDSYIVVGRGEQQINHHWHKSGSSIGKAFITTLKGAQRLA
ALGDTAWDFGVSQVFTSVGKAVHQVFGGAFRSLFGGMSWITQGLLGALLLWGINARDRSIALTFLA
VGGVLLFLSVNVHA
```

Or select file containing sequence(s) No file chosen

Choose a Prediction Method

Prediction Method Show all the method versions:

NetMHCIIpan 4.1 EL (recommended epitope predictor-2023.09) [Help on prediction method selections](#)

Specify what to make binding predictions for

Select species/locus: Human, HLA-DQ

Select MHC allele(s)

Select α & β chains separately if applicable:

Select full HLA reference set:

Select 7-allele HLA reference set:

Allele	
HLA-DRB1*03:01	<input type="checkbox"/>
HLA-DRB1*07:01	<input type="checkbox"/>
HLA-DRB1*15:01	<input type="checkbox"/>
HLA-DRB3*01:01	<input type="checkbox"/>
HLA-DRB3*02:02	<input type="checkbox"/>
HLA-DRB4*01:01	<input type="checkbox"/>
HLA-DRB5*01:01	<input type="checkbox"/>

Select length(s)

default 12-18 as is

11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30

Specify Output

Sort peptides by: Percentile Rank

Output format: XHTML table

Email address (optional):

MHC-II Binding Prediction – Example

tools.iedb.org/mhcii/

MHC-II Binding Prediction Results

Input Sequences

#	Name	Sequence
1	West Nile virus envelope glycoprotein	FNCLGMSNRDFLEGVSGATWDLVLEGGDCVITMSKDKPTIDVKMMNMEA ANLAEVRSYCYLATVSDLSTKAACPTMGEAHNDKRADPAFVCRQGVDRG WGNCGGLFGKGSIDTCAKFACTKAIGRTILKENIKYEVAIFVHGPTTVE SHGNYSTQVGTQAGRFSITPAAPSYTLKLGEGEYVTVDCPRSGIDTNA YYVMTVGTKTFVHREWFMDLNLWSSAGSTVWRNRETLMFEFEPHATKQ SVIALGSQEGALHQAALAGAIPEVFSNTVKLTSGLKCRVKMEKQLKGT TYGVCSKAFKFLGTPADTGHGTWVLELQYTGTDGPKVPISSVASLNDLT PVGRLVTVPFVSVATANAKVLEIEPPFGDSYIVVGRGEQQINHHMKS GSSIGKAFTTTLKGAQRLLAALGDTAHDGFSVGGVFTSVGKAVHQVFGGAF RSLFGGMSWITQGLLGALLWINGINARDRSIALTFLAVGGVLLFLSVNVH A

Prediction method: [netmhciipan_el 4.1](#) | High score = good binders
[Download result](#)

Citations

Allele	#	Start	End	Length	Core Sequence	Peptide Sequence	Score	Percentile Rank
HLA-DQA1*01:01/DQB1*02:01	1	306	320	15	FKFLGTPAD	SKAFKFLGTPADTGH	0.0383	1.30
HLA-DQA1*01:01/DQB1*02:01	1	132	146	15	IKYEVAIFV	KENIKYEVAIFVHG	0.0369	1.40
HLA-DQA1*01:01/DQB1*02:01	1	236	250	15	LMEFEPPHA	RETLMEFEPPHATKQ	0.0305	2.30
HLA-DQA1*01:01/DQB1*02:01	1	305	319	15	FKFLGTPAD	CSKAFKFLGTPADTG	0.0297	2.50
HLA-DQA1*01:01/DQB1*02:01	1	357	371	15	FVSVATANA	TVNPFVSVATANAKV	0.0240	3.90
HLA-DQA1*01:01/DQB1*02:01	1	235	249	15	LMEFEPPHA	NRETLMEFEPPHATK	0.0235	4.10
HLA-DQA1*01:01/DQB1*02:01	1	131	145	15	IKYEVAIFV	LKENIKYEVAIFVHG	0.0233	4.20
HLA-DQA1*01:01/DQB1*02:01	1	237	251	15	LMEFEPPHA	ETLMEFEPPHATKQS	0.0182	7
HLA-DQA1*01:01/DQB1*02:01	1	234	248	15	LMEFEPPHA	RNRETLMEFEPPHAT	0.0181	7.10
HLA-DQA1*01:01/DQB1*02:01	1	307	321	15	FKFLGTPAD	KAFKFLGTPADTGHG	0.0176	7.60
HLA-DQA1*01:01/DQB1*02:01	1	356	370	15	FVSVATANA	VTNPFVSVATANAK	0.0174	7.60
HLA-DQA1*01:01/DQB1*02:01	1	133	147	15	IKYEVAIFV	ENIKYEVAIFVHGPT	0.0156	9.40
HLA-DQA1*01:01/DQB1*02:01	1	304	318	15	FKFLGTPAD	VCSKAFKFLGTPADT	0.0156	9.50
HLA-DQA1*01:01/DQB1*02:01	1	233	247	15	RETLMEFEE	WRNRETLMEFEPPHA	0.0154	9.70
HLA-DQA1*01:01/DQB1*02:01	1	358	372	15	FVSVATANA	VNPFVSVATANAKVL	0.0147	11
HLA-DQA1*01:01/DQB1*02:01	1	224	238	15	SAGSTVWRN	PWSSAGSTVWRNRET	0.0142	12
HLA-DQA1*01:01/DQB1*02:01	1	130	144	15	IKYEVAIFV	ILKENIKYEVAIFVH	0.0141	12
HLA-DQA1*01:01/DQB1*02:01	1	223	237	15	SAGSTVWRN	LPWSSAGSTVWRNRE	0.0138	12
HLA-DQA1*01:01/DQB1*02:01	1	337	351	15	ISSVASLND	KVPISSVASLNDLTP	0.0135	13
HLA-DQA1*01:01/DQB1*02:01	1	43	57	15	MNMEANLA	VKMMNMEANLAEVR	0.0135	13

Input sequence

Output
(sorted high-to-low by score)

Guidelines: Selecting Binders

- Previously, the performance of MHC-II predictions was worse than MHC-I, but this difference has decreased significantly (based on AUC values in automated benchmark)
- Previously established threshold guidelines:
 - Percentile rank ≤ 10.0 (Percentile rank on linear scale (0-100), lower value = better binder)
 - MHC binding affinity $IC_{50} \leq 1000nM$
- **Given the increased prediction performance, threshold can probably be made more stringent – evaluation outstanding**

Issue of Overlapping Peptides

- The tool breaks the sequence into all possible 15-mers - Peptides overlapping by 14 amino acid residues

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	allele	seq_n	start	end	peptide	method	percent	comblib_core	comblib	comblib	smm_align_core	smm_al	smm_al	nn_align_core	nn_a
2	HLA-DPA1*01/DPB1*0401	1	527	541	DHLEFWEGVFTGLTH	Consensus (com	2.52	FWEGVFTGL	6.9	19.59	FWEGVFTGL	310	0.62	EFWEGVFTG	3
3	HLA-DPA1*01/DPB1*0401	1	528	542	HLEFWEGVFTGLTHI	Consensus (com	2.57	FWEGVFTGL	6.9	19.59	FWEGVFTGL	302	0.58	FWEGVFTGL	4
4	HLA-DPA1*01/DPB1*0401	1	526	540	QDHLEFWEGVFTGLT	Consensus (com	2.62	FWEGVFTGL	6.9	19.59	FWEGVFTGL	310	0.62	EFWEGVFTG	4
5	HLA-DPA1*01/DPB1*0401	1	529	543	LEFWEGVFTGLTHID	Consensus (com	3.13	FWEGVFTGL	6.9	19.59	FWEGVFTGL	308	0.61	FWEGVFTGL	5
6	HLA-DPA1*01/DPB1*0401	1	525	539	CQDHLEFWEGVFTGL	Consensus (com	3.26	FWEGVFTGL	6.9	19.59	EFWEGVFTG	320	0.66	FWEGVFTGL	5
7	HLA-DPA1*01/DPB1*0401	1	39	53	AAQTFLATCINGVCW	Consensus (com	3.8	QTFLATCIN	728.23	45.84	FLATCINGV	742	3.36	FLATCINGV	6
8	HLA-DPA1*01/DPB1*0401	1	262	276	GSPITYSTYGKFLAD	Consensus (com	4.07	TYSTYGKFL	2.38	15.24	ITYSTYGKF	827	4.07	ITYSTYGKF	
9	HLA-DPA1*01/DPB1*0401	1	40	54	AQTFLATCINGVCWT	Consensus (com	4.08	QTFLATCIN	728.23	45.84	FLATCINGV	746	3.39	FLATCINGV	7
10	HLA-DPA1*01/DPB1*0401	1	263	277	SPITYSTYGKFLADG	Consensus (com	4.08	TYSTYGKFL	2.38	15.24	ITYSTYGKF	828	4.08	ITYSTYGKF	5
11	HLA-DPA1*01/DPB1*0401	1	38	52	TAAQTFLATCINGVC	Consensus (com	4.13	TAAQTFLAT	52.52	29.77	FLATCINGV	478	1.49	FLATCINGV	7
12	HLA-DPA1*01/DPB1*0401	1	37	51	STAAQTFLATCINGV	Consensus (com	4.56	TAAQTFLAT	52.52	29.77	TAAQTFLAT	464	1.41	FLATCINGV	8
13	HLA-DPA1*01/DPB1*0401	1	261	275	TGSPITYSTYGKFLA	Consensus (com	4.78	TYSTYGKFL	2.38	15.24	ITYSTYGKF	908	4.78	ITYSTYGKF	
14	HLA-DPA1*01/DPB1*0401	1	530	544	EFWEGVFTGLTHIDA	Consensus (com	5	FWEGVFTGL	6.9	19.59	FWEGVFTGL	664	2.75	FWEGVFTGL	9
15	HLA-DPA1*01/DPB1*0401	1	102	116	SDLYLVTRHADVIPV	Consensus (com	7.45	LVTRHADVI	23.49	25.43	YLVRHADV	1194	7.45	YLVRHADV	14
16	HLA-DPA1*01/DPB1*0401	1	41	55	QTFLATCINGVCWTV	Consensus (com	7.57	QTFLATCIN	728.23	45.84	FLATCINGV	829	4.09	FLATCINGV	16
17	HLA-DPA1*01/DPB1*0401	1	101	115	SSDLYLVTRHADVIP	Consensus (com	7.57	LVTRHADVI	23.49	25.43	YLVRHADV	1206	7.57	YLVRHADV	16
18	HLA-DPA1*01/DPB1*0401	1	260	274	TTGSPITYSTYGKFL	Consensus (com	7.71	TYSTYGKFL	2.38	15.24	ITYSTYGKF	1221	7.71	ITYSTYGKF	10
19	HLA-DPA1*01/DPB1*0401	1	100	114	GSSDLYLVTRHADVI	Consensus (com	7.85	GSSDLYLVT	0.74	11.33	YLVRHADV	1183	7.34	YLVRHADV	17
20	HLA-DPA1*01/DPB1*0401	1	531	545	FWEGVFTGLTHIDAH	Consensus (com	7.97	FWEGVFTGL	6.9	19.59	FWEGVFTGL	728	3.24	FWEGVFTGL	17
21	HLA-DPA1*01/DPB1*0401	1	103	117	DLYLVTRHADVIPVR	Consensus (com	8.57	LVTRHADVI	23.49	25.43	YLVRHADV	1307	8.57	YLVRHADV	16

Issue of Overlapping Peptides: Solution

- Post-processing:
 - Remove largely overlapping peptides after prediction (based on same binding core or position)

	A	B	C	D	E	G	H	I	J	K	L	M	N	O
1	allele	seq_n	start	end	peptide	percent	comblib_core	comblib	comblib	smm_align_core	smm_al	smm_al	nn_align_core	nn_a
2	HLA-DPA1*01/DPB1*0401	1	527	541	DHLEFWEGVFTGLTH	2.52	FWEGVFTGL	6.9	19.59	FWEGVFTGL	310	0.62	EFWEGVFTG	3
3	HLA-DPA1*01/DPB1*0401	1	528	542	HLEFWEGVFTGLTHI	2.57	FWEGVFTGL	6.9	19.59	FWEGVFTGL	302	0.58	FWEGVFTGL	4
4	HLA-DPA1*01/DPB1*0401	1	526	540	QDHLEFWEGVFTGLT	2.62	FWEGVFTGL	6.9	19.59	FWEGVFTGL	310	0.62	EFWEGVFTG	4
5	HLA-DPA1*01/DPB1*0401	1	529	543	LEFWEGVFTGLTHID	3.13	FWEGVFTGL	6.9	19.59	FWEGVFTGL	308	0.61	FWEGVFTGL	5
6	HLA-DPA1*01/DPB1*0401	1	525	539	CQDHLEFWEGVFTGL	3.26	FWEGVFTGL	6.9	19.59	EFWEGVFTG	320	0.66	FWEGVFTGL	5
7	HLA-DPA1*01/DPB1*0401	1	39	53	AAQTFLATCINGVCW	3.8	QTFLATCIN	728.23	45.84	FLATCINGV	742	3.36	FLATCINGV	6
8	HLA-DPA1*01/DPB1*0401	1	262	276	GSPITYSTYGKFLAD	4.07	TYSTYGKFL	2.38	15.24	ITYSTYGKF	827	4.07	ITYSTYGKF	
9	HLA-DPA1*01/DPB1*0401	1	40	54	AQTFLATCINGVCWT	4.08	QTFLATCIN	728.23	45.84	FLATCINGV	746	3.39	FLATCINGV	7
10	HLA-DPA1*01/DPB1*0401	1	263	277	SPITYSTYGKFLADG	4.08	TYSTYGKFL	2.38	15.24	ITYSTYGKF	828	4.08	ITYSTYGKF	5

T Cell – MHC Class II Binding Prediction

tools.iedb.org/main/tcell/

IEDB Analysis Resource

Overview | **T Cell Tools** | B Cell Tools | Analysis Tools | Tools-API | Usage | Download | Datasets | Contribute Tools | References

T Cell Epitope Prediction Tools

T Cell Epitopes - MHC Binding Prediction

These tools predict IC50 values for peptides binding to specific MHC molecules. Note that binding to MHC is necessary but not sufficient for recognition by T cells.

[Peptide binding to MHC class I molecules](#)
This tool will take in an amino acid sequence, or set of sequences and determine each subsequence's ability to bind to a specific MHC class I molecule.

[Peptide binding to MHC class II molecules](#)
This tool employs different methods to predict MHC Class II epitopes, including a consensus approach which combines NN-align, SMM-align and Combinatorial library methods.

TepiTool:
The Tepitool provides prediction of peptides binding to MHC class I and class II molecules. Tool is designed as a wizard with 6 steps as described below. Each field (except sequences and alleles) is filled with default recommended settings for prediction and selection of optimum peptides. The input parameters can be adjusted as per your specific needs. You can go back to previous steps to change your selection before submission of the job. Once you submit the job (at the end of step-6), you will not be able to make any more changes and will have to start the prediction all over again with updated input parameters.

T Cell Epitopes - Processing Prediction

These tools predict epitope candidates based upon the processing of peptides in the cell.

[Proteasomal cleavage/TAP transport/MHC class I combined predictor](#)
This tool combines predictors of proteasomal processing, TAP transport, and MHC binding to produce an overall score for each peptide's intrinsic potential of being a T cell epitope.

[Neural network based prediction of proteasomal cleavage sites \(NetChop\) and T cell epitopes \(NetCTL and NetCTLpan\)](#)
NetChop is a predictor of proteasomal processing based upon a neural network. NetCTL and NetCTLpan are predictors of T cell epitopes along a protein sequence. It also employs a neural network architecture.

[MHC-NP: Prediction of peptides naturally processed by the MHC](#)
MHC-NP employs data obtained from MHC elution experiments in order to assess the probability that a given peptide is naturally processed and binds to a given MHC molecule. This tool was the winner of the [2nd Machine Learning Competition in Immunology](#).

MHCII-NP:
This tool utilizes MHC II ligand elution data to predict naturally processed MHC II ligands by scanning the given peptide sequences.

T Cell Epitopes - Immunogenicity Prediction

This tool predicts the relative ability of a peptide/MHC complex to elicit an immune response.

[T cell class I pMHC immunogenicity predictor](#)
This tool uses amino acid properties as well as their position within the peptide to predict the immunogenicity of a class I peptide MHC (pMHC) complex.

Deimmunization:
The deimmunization tool is attempt to identify immunodominant regions in a given therapeutically important protein, and suggest amino-acid substitutions that create non-immunogenic versions of the proteins. So we have opted a two steps process; 1) In the



Step 1: Sequence Data

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TepiTool

Steps **1** 2 3 4 5 6

SEQUENCE - Provide sequence data:

Enter sequence(s) in FASTA or PLAIN format.

```
>Seq_1
MKALIVLGLVLLSVTVQGKVFCEARTLKRLGMDGYRGISLANWMCLAKW
>Seq_2
MLLALVCLLSCLANSDF
>Seq_3
MKALIVLGLVLLSVTVQGKVFERCELAR
```

FASTA format detected.

Or upload file containing sequence(s) No file chosen

Step 2: Species & Allele Class

tools.iedb.org/tepitool/

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TepiTool

Steps 1 **2** 3 4 5 6

SPECIES & ALLELE CLASS - Select the host species and MHC allele class:

Host species	Human
Allele class	

Start Over Back

Current selections:
No. of sequences 3

Step 2: Species & Allele Class

tools.iedb.org/tepitool/

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TepiTool

Steps 1 **2** 3 4 5 6

SPECIES & ALLELE CLASS - Select the host species and MHC allele class:

Host species	Human
Allele class	Class I Class I Class II

Start Over Back

Current selections:
No. of sequences 3

Step 3: Allele Selection

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TepiTool

Steps 1 2 3 4 5 6

ALLELES - Specify alleles:

Human - Class I

- Select from list of frequently occurring alleles (Frequency > 1%)
- Select from list of all available alleles
- Select from list of representative alleles from different HLA supertypes
- Use panel of 27 most frequent A & B alleles
- Upload allele file

Alleles

- A*01:01
- A*02:01
- A*02:06
- A*03:01
- A*11:01
- A*23:01
- A*24:02
- A*25:01
- A*26:01
- A*29:02
- A*30:01
- A*30:02

Start Over Back Next

Current selections:	
No. of sequences	3
Host species	Human
Allele class	Class I
Selected alleles	1.A*02:01 2.A*02:06 3.A*03:01



Step 4: Peptides - Class I

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TepiTool

Steps 1 2 3 **4** 5 6

PEPTIDES - Select peptides to be included in prediction:

Peptides to be included in prediction	<input checked="" type="radio"/> Apply default settings for low number of peptides <input type="radio"/> Apply default settings for moderate number of peptides <input type="radio"/> Apply default settings for high number of peptides <input type="radio"/> Custom selection - Select your own settings
	Handling of duplicate peptides: - Duplicate peptides will be removed.
	Peptide lengths to be considered in prediction: - Only peptide length 9 will be included 9mers = 58
Conservancy analysis (Uses only peptides conserved in specified % of sequences)	<input checked="" type="radio"/> No <input type="radio"/> Yes

[Start Over](#) [Back](#) [Next](#)

Current selections:	
No. of sequences	3
Host species	Human
Allele class	Class I
Selected alleles	1.A*02:01 2.A*02:06 3.A*03:01

Step 4: Peptides - Class I

tools.iedb.org/tepitool/

IEDB Analysis Resource

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TepiTool

Steps 1 2 3 **4** 5 6

PEPTIDES - Select peptides to be included in prediction:

Apply default settings for low number of peptides
 Apply default settings for moderate number of peptides
 Apply default settings for high number of peptides
 Custom selection - Select your own settings

Handling of duplicate peptides:
 Remove duplicate peptides
 Keep duplicate peptides

Peptide lengths to be considered in prediction:
 8mers = 60
 9mers = 58
 10mers = 56
 11mers = 54
 12mers = 52
 13mers = 50
 14mers = 48

Peptides to be included in prediction

Conservancy analysis
(Uses only peptides conserved in specified % of sequences)
 No
 Yes

Use peptides conserved in

[Start Over](#) [Back](#) [Next](#)

Current selections:

No. of sequences	3
Host species	Human
Allele class	Class I
Selected alleles	1.A*02:01 2.A*02:06 3.A*03:01

1 sequence
10% sequences
20% sequences
30% sequences
40% sequences
50% sequences
60% sequences
70% sequences
80% sequences
90% sequences
100% sequences

Step 5: Method - Class I

IEDB Analysis Resource

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TepiTool

Steps 1 2 3 4 5 6

METHOD - Select prediction & peptide selection methods and cutoff values:

Prediction method to use

IEDB recommended

Selection of predicted peptides

Select peptides based on predicted percentile rank

Select peptides with predicted consensus percentile rank \leq 1

Start Over Back Next

- IEDB recommended
- Consensus
- NetMHCpan
- ANN
- SMMPMBEC
- SMM
- ComLib_Sidney_2008

Current selections:

No. of sequences	3
Host species	Human
Allele class	Class I
Selected alleles	1.A*02:01 2.A*02:06 3.A*03:01
Duplicate peptides	Removed
Peptide lengths selected	9mers 10mers
No. of peptides included (Not considering conservancy analysis)	114
Conservancy analysis	Peptides conserved in at least 50% sequences

Step 5: Method - Class I

tools.iedb.org/tepitool/

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TepiTool

Steps 1 2 3 4 5 6

METHOD - Select prediction & peptide selection methods and cutoff values:

Prediction method to use

IEDB recommended

Selection of predicted peptides

Select peptides based on predicted percentile rank

Select peptides with predicted consensus percentile rank \leq 1

Start Over Back Next

Current selections:

No. of sequences	3
Host species	Human
Allele class	Class I
Selected alleles	1.A*02:01 2.A*02:06 3.A*03:01
Duplicate peptides	Removed
Peptide lengths selected	9mers 10mers
No. of peptides included (Not considering conservancy analysis)	114
Conservancy analysis	Peptides conserved in at least 50% sequences

Select peptides based on predicted percentile rank
Select peptides based on predicted IC50
Select peptides based on MHC specific predicted binding threshold*
Select top x% of predicted peptides**
Select top x number of predicted peptides**

Articles in this section

[Population coverage issue: genotypic frequency is currently not working as expected](#)

[HLA nomenclature](#)

[Introduction and tutorials](#)

[MHC class I binding prediction - Internal Server Error](#)

[MHC II Epitope Prediction - "Internal Server Error"](#)

[When can I consider an epitope as non-binder using MHC class I and II binding predictions tools?](#)

[HLA allele frequencies and reference sets with maximal population coverage](#)

[Selecting thresholds \(cut-offs\) for MHC class I and II binding predictions](#)

[T Cell Epitopes - MHC Class II Binding Prediction Tools Description](#)

[T Cell Epitopes - MHC Class I Binding Prediction Tools Description](#)

Selecting thresholds (cut-offs) for MHC class I and II binding predictions



Permanently deleted user

10 months ago · Updated

Follow

The predicted binding affinities and ranks that result from the IEDB prediction tools should be treated as ranking metrics as a way to prioritize peptides for experimental testing. There are many ways to rank the peptides. Here, we list only the latest recommendations.

MHC class I

For MHC class I T cell epitope predictions, the latest research from our group¹ shows that setting a common threshold for eluted ligand (EL) rank of ~1.1 in NetMHCpan 4.0 across all alleles results in 80% sensitivity for capturing immunogenic peptides. Allele-specific thresholds have also been established and are contained within the **supplemental data** of the paper. However, the increase in sensitivity and specificity is marginal unless there are relatively few alleles being considered with very divergent thresholds.

MHC class II

For MHC class II T cell epitope predictions, selection of predicted binders can be done based on the percentile rank or MHC binding affinity. The IEDB currently recommends making selections based on a consensus percentile rank of the top 20%², which captures 50% of the immune response. Alternatively, selecting peptides predicted to bind at 1,000nM is also supported by experimental data³.

1. Reardon et al., 2021 Mol. Cell. Proteomics (PMID 34303001)
2. Paul et al., 2015 J. Imm. Methods (PMID 25862607)
3. Southwood et al., 1998, J. Immunology (PMID 9531296)

<https://help.iedb.org/hc/en-us/articles/114094151811-Selecting-thresholds-cut-offs-for-MHC-class-I-and-II-binding-predictions>

Step 5: Method - Class I

Steps 1 2 3 4 **5** 6

METHOD - Select prediction & peptide selection methods and cutoff values:

Prediction method to use: IEDB recommended

Select peptides based on MHC specific predicted binding t

(*Each MHC allele has its own IC50 threshold. Predicted peptides will correspond to 75% of immune response. Prediction method is SMM)

As of now, only the following alleles are covered by this method:

- A*01:01
- A*02:01
- A*02:03
- A*02:06
- A*03:01
- A*11:01
- A*23:01
- A*24:02
- A*25:01
- A*26:01
- A*29:02
- A*30:01
- A*30:02
- A*31:01
- A*32:01
- A*33:01
- A*68:01
- A*68:02
- B*07:02
- B*08:01
- B*14:02
- B*15:01
- B*18:01
- B*27:05
- B*35:01
- B*35:03
- B*38:01
- B*39:01
- B*40:01
- B*40:02
- B*44:02
- B*44:03
- B*46:01
- B*48:01
- B*51:01
- B*53:01
- B*57:01
- B*58:01

Selection of predicted peptides

Please refer this paper for more details: [Paul et al. \(2013\)](#), J of Immunol. 191(12): 5831-5839.

Start Over Back Next

Current selections:	
No. of sequences	3
Host species	Human
Allele class	Class I
Selected alleles	1. A*02:01 2. A*02:06 3. A*03:01
Duplicate peptides	Removed
Peptide lengths selected	9mers 10mers
No. of peptides included (Not considering conservancy analysis)	114
Conservancy analysis	Peptides conserved in at least 50% sequences

Step 5: Review & Submit

tools.iedb.org/tepitool/

TepiTool

Steps 1 2 3 4 5 **6**

REVIEW: Review selections, enter job details & submit data:

Summary:

No. of sequences	3
Host species	Human
Allele class	Class I
Alleles	1.A*02:01 2.A*02:06 3.A*03:01
Duplicate peptides	Removed
Peptide lengths selected	9mers 10mers
Approx no. of peptides included	114
Peptide overlap	N/A (all possible nmers are included in class I)
Conservancy analysis	Peptides conserved in at least 50% sequences
Prediction method	IEDB recommended
Peptide selection criterion	Based on predicted consensus percentile rank (Cutoff selected = 1)

Job details:

Job name (optional)	<input type="text"/>
Email (optional - will notify when job is finished)	<input type="text" value="bpeters@lji.org"/>

(Please note that you will not be able to make any more changes once submitted. You will have to start again if you want to do so.)

Step 4: Peptides - Class II

Repeat the prediction for class II alleles

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TepiTool

Steps: 1 | 2 | 3 | **4** | 5 | 6

PEPTIDES - Select peptides to be included in prediction:

Apply default settings for low number of peptides
 Apply default settings for moderate number of peptides
 Apply default settings for high number of peptides
 Custom selection - Select your own settings

Peptides to be included in prediction

Handling of duplicate peptides
- Duplicate peptides will be removed.

Desired no. of overlapping residues for 15mers
- No. of overlapping residues fixed at 10.

Approximate no. of peptides to be considered for prediction = 12

Conservancy analysis (Uses only peptides conserved in specified % of sequences)
 No
 Yes
 Use peptides conserved in

settings summary →

	Low	Moderate	High	Custom
Duplicates	removed	removed	not removed	user selects
Overlapping residues	8	10	10	user selects
Approx. # peptides	10	12	14	12

Current selections:

No. of sequences	3
Host species	Human
Allele class	Class II
Selected alleles	1.DPA1*01:03/DPB1*02:01 2.DQA1*03:01/DQB1*03:02 3.DRB1*01:01 4.DRB1*01:02 5.DRB1*01:03

Step 5: Method - Class II

IEDB Analysis Resource

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TepiTool

Steps 1 2 3 4 **5** 6

METHOD - Select prediction & peptide selection methods and cutoff values:

Prediction method to use	IEDB recommended ▾
Selection of predicted peptides	Select peptides based on predicted percentile rank ▾ Select peptides with predicted consensus percentile rank ≤ <input type="text" value="10"/>

Start Over Back Next

IEDB recommended
Consensus
NetMHCIIpan
NN_align
SMM_align
Combinatorial library
Sturniolo

Current selections:	
No. of sequences	3
Host species	Human
Allele class	Class II
Alleles selected	1.DPA1*01:03/DPB1*02:01 2.DQA1*03:01/DQB1*03:02 3.DRB1*01:01 4.DRB1*01:02 5.DRB1*01:03
Duplicate peptides	Removed
Peptide overlap	10 AA residues
Approx no. of peptides included (Not considering conservancy analysis)	12
Conservancy analysis	Peptides conserved in at least 50% sequences

Step 5: Method - Class II

IEDB Analysis Resource

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TepiTool

Steps 1 2 3 4 **5** 6

METHOD - Select prediction & peptide selection methods and cutoff values:

Prediction method to use

IEDB recommended ▾

Selection of predicted peptides

Select peptides based on predicted percentile rank ▾

Select peptides with predicted consensus percentile rank \leq

[Start Over](#) [Back](#) [Next](#)

Current selections:

No. of sequences	3
Host species	Human
Allele class	Class II
Alleles selected	1.DPA1*01:03/DPB1*02:01 2.DQA1*03:01/DQB1*03:02 3.DRB1*01:01 4.DRB1*01:02 5.DRB1*01:03
Duplicate peptides	Removed
Peptide overlap	10 AA residues
Approx no. of peptides included (Not considering conservancy analysis)	12
Conservancy analysis	Peptides conserved in at least 50% sequences

Step 6: Review & Submit

tools.iedb.org/tepitool/

TepiTool

Steps 1 2 3 4 5 **6**

REVIEW: Review selections, enter job details & submit data:

Summary:

No. of sequences	3
Host species	Human
Allele class	Class II
Alleles	1.DPA1*01:03/DPB1*02:01 2.DQA1*03:01/DQB1*03:02 3.DRB1*01:01 4.DRB1*01:02 5.DRB1*01:03
Duplicate peptides	Removed
Peptide lengths selected	15mers (Only one length for class II)
Approx no. of peptides included	12
Peptide overlap	10 AA residues
Conservancy analysis	Peptides conserved in at least 50% sequences
Prediction method	IEDB recommended
Peptide selection criterion	Based on predicted consensus percentile rank (Cutoff selected = 10)

Job details:

Job name (optional)	<input type="text"/>
Email (optional - will notify when job is finished)	<input type="text" value="bpeters@lji.org"/>

(Please note that you will not be able to make any more changes once submitted. You will have to start again if you want to do so.)

Step 5: Method - Class II

Alternative Methods

IEDB Analysis Resource

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TepiTool

Steps 1 2 3 4 5 6

METHOD - Select prediction & peptide selection methods and cutoff values:

Prediction method to use: IEDB recommended

Selection of predicted peptides: Select peptides based on predicted percentile rank

Select peptides with predicted consensus percentile rank \leq 10

Start Over Back Next

Current selections:

No. of sequences	3
Host species	Human
Allele class	Class II
Alleles selected	1.DPA1*01:03/DPB1*02:01 2.DQA1*03:01/DQB1*03:02 3.DRB1*01:01 4.DRB1*01:02 5.DRB1*01:03
Duplicate peptides	Removed
Peptide overlap	10 AA residues
Approx no. of peptides included (Not considering conservancy analysis)	12
Conservancy analysis	Peptides conserved in at least 50% sequences

- Select peptides based on predicted percentile rank
- Select peptides based on predicted IC50
- Select peptides based on predicted no. of alleles binding
- Select top x% of peptides*
- Select top x number of predicted peptides*

exclusive to class II

Select peptides based on predicted no. of alleles binding

Select peptides that bind to at least 50% alleles
(binding determined by IEDB consensus percentile rank \leq 20.0)

Step 3-5: Class II – 7 Allele Method

tools.iedb.org/tepitool/

Steps 1 2 **3** 4 5 6

ALLELES - Specify alleles:

Human - Class II

Predict for custom allele set
 Predict for pre-selected panel of alleles
 Predict using pre-selected allele sets & methods

Alleles

Options:

Use the "7-allele method"
 Use panel of 26 most frequent alleles for promiscuous binding

- Selection criterion is median of percentile ranks from the 7 alleles involved.

Current selections:	
No. of sequences	3
Host species	Human
Allele class	Class II
Selected alleles	1. DRB1*03:01 2. DRB1*07:01 3. DRB1*15:01 4. DRB3*01:01 5. DRB3*02:02 6. DRB4*01:01 7. DRB5*01:01

[Reset alleles](#)

Steps 1 2 3 **4** 5 6

PEPTIDES - Select peptides to be included in prediction:

Handling of duplicate peptides	Duplicate peptides will be removed
No. of overlapping residues for 15mer peptides to be generated (Peptide length is fixed at 15 for class II)	10
Approximate no. of peptides to be considered for prediction	12

Start Over Back Next

Steps 1 2 3 4 **5** 6

METHOD - Select prediction & peptide selection methods and cutoff values:

Prediction method to use	IEDB recommended
Selection of predicted peptides	Promiscuity based on "7-allele method"* - Peptides considered as binders if median consensus percentile \leq 20

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T Cell Summary

MHC Class II Binding Prediction

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T Cell – MHC Class II Processing Prediction

tools.iedb.org/main/tcell/

IEDB Analysis Resource

Overview | **T Cell Tools** | B Cell Tools | Analysis Tools | Tools-API | Usage | Download | Datasets | Contribute Tools | References

T Cell Epitope Prediction Tools

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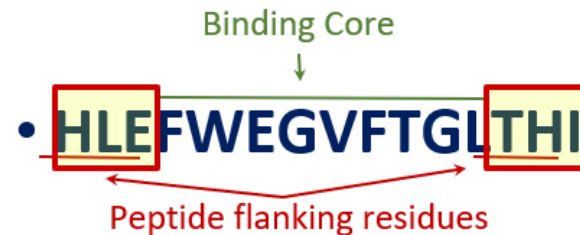
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MHC-II peptide processing

MHC-II naturally processed peptides are generated by proteases, but the ends typically flank the binding core, and don't contribute to binding




MHCII-NP

- Predicting the naturally processed peptides for MHC class II
- Based on
 - Cleavage motif analysis at C and N terminal of peptides
 - Ligand elution data derived from IEDB
- Ligand predictions is improved markedly when combining the binding and cleavage motifs
- T cell epitope prediction is not significantly improved

MHCII-NP – Example

<http://tools.iedb.org/mhciinp/>

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MHCII-NP: Prediction of naturally processed MHC II ligands

Sequences

Enter sequences in FASTA or plain format

```
>sp|P15848|ARSB_HUMAN Arylsulfatase B OS=Homo sapiens OX=9606
GN=ARSB PE=1 SV=1
MGPRGAASLPRGPGPRRLLLPVVLPLLLLLLLAPPGSAGASRPPHLVFLADDLGNWV
GFHGSRI RTPHLDALAAGVLLDNYTQPLCTPSRSQLLTGRYQIRTGLQHQIIWPCQPS
CVPLDEKLLPQLLKEAGYTTMVGKWHLGMYRKECLPTRRGFDYFGYLLGSEDYYSHER
CTLIDALNVTRCALDFRDGEEVATGYKNMYSTNIFTKRAIALITNHPPEKPLFLYLALQS
VHEPLQVPEEYLKPYDFIQDKNRHHYAGMVSLMDEAVGNVTAALKSSGLWNNTVFI FSTD
NGGQTLAGGNNWPLRGRKWSLWEGGVRGVGFVAPLLKQKGVKNRELIHISDWLPTLVKL
ARGHTNGTKPLDGFVWKTISEGSPSPRIELLNIDPNFVDS SPCPRNSMAPAKDDSSLP
EYSAFN TSVHAAIRHGNWKL LTGYPGCGYWFP PPSQYNVSEIPSSDPPTKTLWLFIDIRD
PEERHDL SREYPHIVTKLLSRLQFYHKHSVPVYFPAQDPRCDPKATGVWGPWM
```

Or upload sequences as a text file No file chosen



MHCII-NP – Example

<http://tools.iedb.org/mhciinp/>

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MHCII-NP results

Top 5 peptides per input sequence:

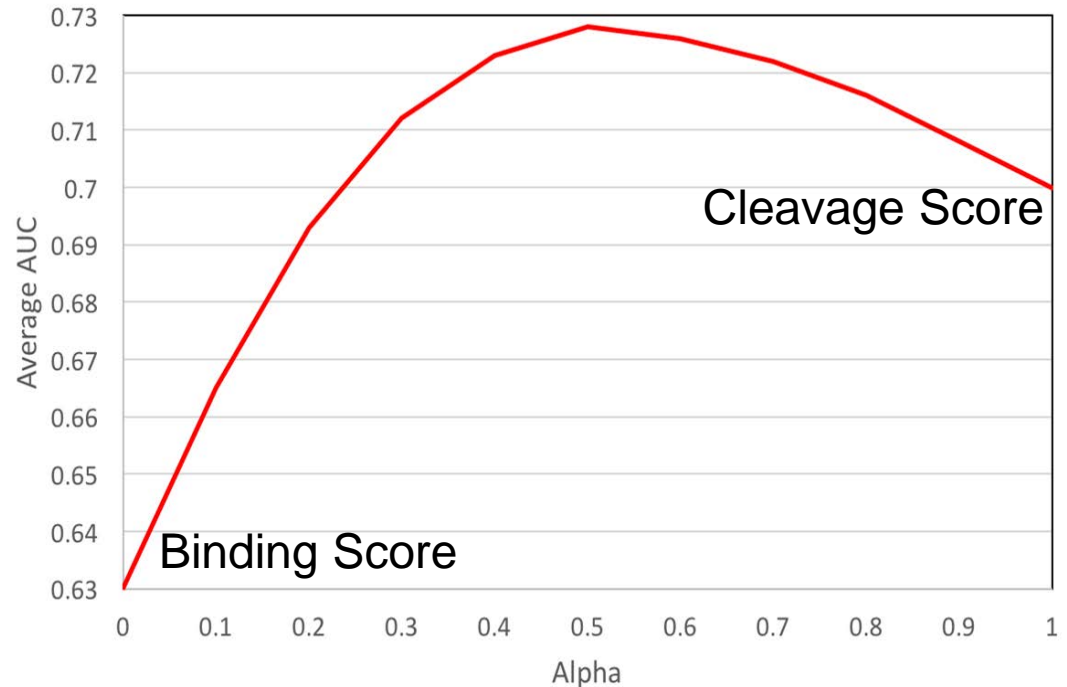
	Seq name	Peptide start	Peptide end	Peptide length	Peptide	N motif	C motif	Cleavage probability score	Cleavage probability percentile rank
1	SPIP15848 ARSB_HUMAN ARYLSULFATASE_B OS=HOMO S...	510	524	15	VPVYFPAQDPRCDPK	SVP	PKA	1.75814	0.00
2	SPIP15848 ARSB_HUMAN ARYLSULFATASE_B OS=HOMO S...	2	16	15	GPRGAASLPRGPGPR	MGP	PRR	1.73735	0.02
3	SPIP15848 ARSB_HUMAN ARYLSULFATASE_B OS=HOMO S...	247	261	15	VPEEYLKPYDFIQDK	QVP	DKN	1.48840	0.04
4	SPIP15848 ARSB_HUMAN ARYLSULFATASE_B OS=HOMO S...	384	398	15	SPSPRIELLHNIDPN	GSP	PNF	1.40420	0.05
5	SPIP15848 ARSB_HUMAN ARYLSULFATASE_B OS=HOMO S...	12	26	15	GPGPRRLLLPVVLPL	RGP	PLL	1.33714	0.07

[complete results \(TSV\)](#)

MHCII-NP Scores

- **Cleavage Score:** Derived from the cleavage motif analysis in ligand elution data
- **Binding Score:** Derived from HLA binding affinity using 7-allele method (Paul et. al. 2015).

$$\text{Combined score} = \alpha \times \text{cleavage probability score} + (1 - \alpha) \times \text{binding score}$$



Barra et al. *Genome Medicine* (2018) 10:84
<https://doi.org/10.1186/s13073-018-0594-6>

Genome Medicine

RESEARCH

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Footprints of antigen processing boost MHC class II natural ligand predictions

Carolina Barra^{1*}, Bruno Alvarez^{2,1*}, Sinu Paul², Alessandro Sette², Bjoern Peters², Massimo Andreatta¹, Soren Buus³ and Morten Nielsen^{1,4*}

Front Immunol. 2018 Aug 6;9:1795. doi: 10.3389/fimmu.2018.01795. eCollection 2018.

Determination of a Predictive Cleavage Motif for Eluted Major Histocompatibility Complex Class II Ligands.

Paul S¹, Karosiene E¹, Dhanda SK¹, Jurtz V², Edwards L¹, Nielsen M^{2,3}, Sette A^{1,4}, Peters B^{1,4}.

PMID: 30127785 PMCID: [PMc6087742](https://pubmed.ncbi.nlm.nih.gov/30127785/) DOI: [10.3389/fimmu.2018.01795](https://doi.org/10.3389/fimmu.2018.01795)

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[CD4 T cell immunogenicity prediction:](#)

The server is developed to predict the allele independent CD4 T cell immunogenicity at population level. User can predict the T cell immunogenicity using 7-allele method ([Paul et. al. 2015](#)), immunogenicity method and combined method (IEDB recommended). The combined method predicts the final score that combines the predictions from 7-allele method and immunogenicity method.



MHC-II Restricted Immunogenicity Prediction

- Extracted datasets of proteins from the IEDB for which overlapping peptides were tested for immunogenicity
- Utilized these datasets to train a Neural Network to learn 'motifs' associated with immunogenicity independent of specific MHC alleles expressed
- Resulting score can be combined with '7 allele method' quantifying MHC binding across alleles to predict overall immunogenicity


Class II Immunogenicity Prediction

- Based on Neural network model trained on
 - In house dataset for different antigens tested on different population cohorts
 - Tetramer dataset- derived from IEDB
- Validated on 57 independent studies from different groups across the world
- Implemented three approaches
 - 7-allele method (*Paul et. al. 2015*)
 - Immunogenicity predictions
 - Hybrid approach

Class II Immunogenicity Prediction – Example

<http://tools.iedb.org/CD4episcore/>

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CD4 T cell immunogenicity prediction


Specify Sequence(s)

Enter epitope sequence(s) in PLAIN or FASTA format


```
>sp|P01588|EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1
MGVHECPAWLWLLLSLLSLPLGLPVLGAPPRILICDSRVLERYLLEAKEAENITTGCAEHC
SLNENITVPDTKVNIFYAWKRMEVGGQAVEVWQGLALLSEAVLRGQALLVNSSQPWEPLQL
HVDKAVSGLRSLTLLRALGAQKEAISPPDAASAAPLRTITADTFRKLFRVYSNFLRGKL
KLYTGEACRTGDR
```


Or upload epitope sequence(s) from a file No file chosen

Choose a prediction method

Prediction method: 

Specify Output

Sort Peptides by: 

Select maximum combined score threshold: 

Enter the Job Name (Optional)

Email address (optional)

Class II Immunogenicity Prediction – Example

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CD4 Immunogenicity prediction results

Number of proteins: 1

Number of 15mer (overlapping 10mer): 37

Threshold : 50.0%

Method : combined

[Download result](#)

[Citations](#)

Protein Number	Protein Description	Peptide	Start	End	Combined Score	Immunogenicity Score	Peptide core	Median Percentile Rank (7-allele)	HLA-DRB1:03:01	HLA-DRB1:07:01	HLA-DRB1:15:01	HLA-DRB3:01:01	HLA-DRB3:02:02	HLA-DRB4:01:01	HLA-DRB5:01:01
1	sp P01588 EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1	WLLLSLLSLPLGLPV	11	25	42.16452	95.0613	LLSLLSLPL	6.9	25.0	3.2	3.6	73.0	33.0	6.9	6.5
1	sp P01588 EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1	TKVNFYAWKRMEVGQ	71	85	47.39488	67.4872	TKVNFYAWK	34.0	52.0	22.0	15.0	71.0	30.0	65.0	34.0
1	sp P01588 EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1	EPLQLHVDKAVSGLR	116	130	32.55636	43.8909	LHVDKAVSG	25.0	5.4	59.0	40.0	22.0	7.0	38.0	25.0
1	sp P01588 EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1	VSGLRSLTLLRALG	126	140	44.95964	86.8991	LTTLLRALG	17.0	12.0	17.0	9.3	70.0	20.0	20.0	1.3
1	sp P01588 EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1	SLTLLRALGAQKEA	131	145	42.78744	69.4686	LLRALGAQK	25.0	47.0	46.0	21.0	89.0	25.0	14.0	1.6
1	sp P01588 EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1	PLRTITADTFRKLF	156	170	46.18064	85.4516	LRTITADTF	20.0	6.1	47.0	40.0	8.0	20.0	20.0	20.0
1	sp P01588 EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1	TADTFRKLFVYSNF	161	175	46.66984	44.6746	FRKLFVYS	48.0	63.0	58.0	23.0	48.0	33.0	53.0	24.0
1	sp P01588 EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1	RKLFRVYSNFLRGK	166	180	13.8966	22.4415	FRVYSNFLR	8.2	53.0	8.2	0.12	27.0	4.8	26.0	1.5

[Download result](#)

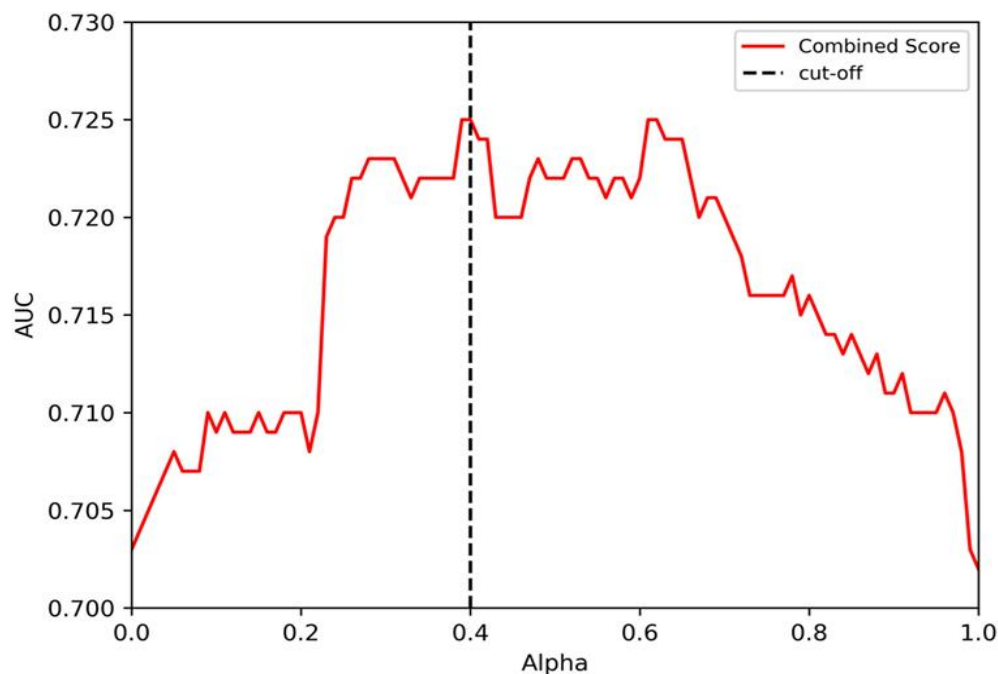
[Citations](#)

If you use CD4 T cell Immunogenicity prediction tool in a manuscript, please cite following article:
 Dhanda et. al.: Predicting HLA CD4 immunogenicity in human populations; *Frontiers in Immunology*, 2018, 9, 1369 [Click here to read full text](#)

Class II Immunogenicity Prediction Scores

- Immunogenicity Score: Derived from the neural network model trained on Immunogenicity data
- HLA Score: Derived from HLA binding affinity using 7-allele method (Paul et. al. 2015).

$$\text{Combined score} = \alpha \times \text{Imm score} + (1 - \alpha) \times \text{HLA score.}$$



[Front Immunol.](#) 2018 Jun 14;9:1369. doi: 10.3389/fimmu.2018.01369. eCollection 2018.

Predicting HLA CD4 Immunogenicity in Human Populations.

[Dhanda SK](#)¹, [Karosiene E](#)¹, [Edwards L](#)¹, [Grifoni A](#)¹, [Paul S](#)¹, [Andreatta M](#)², [Weiskopf D](#)¹, [Sidney J](#)¹, [Nielsen M](#)^{2,3}, [Peters B](#)^{1,4}, [Sette A](#)^{1,4}.

PMID: 29963059 PMCID: [PMC6010533](#) DOI: [10.3389/fimmu.2018.01369](#)

Class II Summary

- Performance of MHC II binding- and ligand elution predictions has massively improved in the last years; (thresholds have not yet been updated to reflect that)
- NetMHCIIpan 4.1 BA scores should be used for binding affinity and EL scores for ligand elution and epitope predictions
- The promiscuous nature of MHC-II binding motifs and ability to bind in different registers of a peptide allows identifying broadly reactive epitopes (→ 7 allele method)
- Tepitool implements pre-and post-processing steps for T cell epitope prediction for both class I and class II – and should be a good starting point

Both class I and II epitope predictions are constantly being re-evaluated, and all of these recommendations are subject to change