

T Cell processing & immunogenicity predictions

tools.iedb.org

Presented by: Dr. Bjoern Peters, Professor

2022 IEDB User Workshop

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CD8+ T cell epitopes in viral infection



CD8+ T cell epitopes in viral infection



TE 11 ORFT MGQIVIMFEALPHIIDEVINIVIIVLIV	<u>/ G K A V Y N</u>
I CEII ORF2 <u>MGLKGPDIYKGVYQFKSVEFDMSHLNLT</u>	ГМРNACSANN
ORF3 MHNFCNLTSAFNKKTFDHTLMSIVSSLH	<u>ILSIDGNSNY</u>
CPHOPE ORF4 MSAQSQCRTFRGRVLDMFRTAFGGKYMR	<u>R S G W G W T G S D</u>
manning ORF5 MHCTYAGPFGMSRILLSQEKTKFFTRL	_ AGTFTWTLS
mapping ORF6 MKCFGNTAVAKCNVNHDAEFCDMLRLID) Y N K A A L S K F
ORF7 <u>MLMRNHLLDLMGVPYCNY</u> SKFWYLEHAK	<u>(ТGЕТЅVРКС</u>

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MHC I - Antigen processing and presentation pathway



Peters et al, J Mol Biol 2002, Bioinformatics 2003, J Immunol. 2003; CMLS 2005; Assarson, J Immunol 2007

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Class I Processing + immunogenicity tools available in the IEDB <u>http://tools.iedb.org/main/tcell/</u>

IEDB Analysis Resource

	Overview	T Cell Tools	B Cell Tools	Analysis Tools	Tools-API	Usage	Download	Datasets	Contribute Tools	References		
		Epitopes -	Processing	g Prediction								
	These too	ols predict ep	itope candida	ites based upon	the proces	sing of p	eptides in ti	ne cell.				•
ľ	Proteaso	mal cleavage	e/TAP transpo	ort/MHC class I	combined	predictor			inding to produ		anara far anah	L
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	epito	opes along a	protein sequ	ience. It also e	mploys a r	neural ne	etwork arch	itecture.				L
Ī	MHC-NP	P: Prediction of	of peptides na	aturally process	ed by the N	<u>1HC</u>						L
	MHC	C-NP employs	s data obtain	ed from MHC e	lution expe	eriments	in order to	assess th	e probability tł Machine Learni	nat a given pe	ptide is naturally	L
-	proc	coocu unu p			ne. mis to	or was a	ie winner e	1 the <u>2nd 1</u>		ng competitie	<u>in in initiatiology</u> .	۰.
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Class I 'combined predictor'

Home Help Example Reference	Download Contact
MHC-I Processing Pr	adictions
Prediction Method Version	2013-02-22 [Older versions]
	Specify Sequence(s)
Enter protein sequence(s) in FASTA format (Browse for sequences in NCBI)	a.
Or select file containing sequence(s)	Browse No file selected.
Choose sequence format	auto detect format
	Choose a Prediction Method
Prediction Method	IEDB recommended V Help on prediction method selections
	Specify what to make binding predictions for
MHC source species	human 🗸
Show only frequently occuring alleles: 🗹 🔅 Select MHC allele(s)	Allele Lenath Upload allele file ?
	Proteasomal cleavage prediction
Specify proteasome type	immuno 🔽
	TAP transport predictions
Maximum precursor extension	1
Alpha factor	0.2
	Specify Output
Output format	XHTML table v
	Submit Reset

- Combines predictions for:
 - proteasomal cleavage
 - TAP transport
- Trained on specific in vitro datasets

Proteasomal cleavage

	Proteasomal cleavage prediction		
Specify proteasome type	immuno 🔽	~	
	TAP transport predictions	immuno	
Maximum precursor extension	1	constitutive	
Alpha factor	0.2		

- Proteasomes create the C-terminal end of peptides
- Prediction looks for sequence motive up and downstream of potential cleavage site
- Cleavage sequence motif determined based on in vitro protein digests by proteasomes

TAP Transport

	Proteasomal cleavage prediction
Specify proteasome type	immuno
	TAP transport predictions
Maximum precursor extension	1
Alpha factor	0.2

- TAP transport efficiency of peptides is sequence dependent; motif derived based on in vitro assays
- Overall TAP transport efficiency of a presented MHC ligand can be result of a collection of precursors
- Unless paper specifically read and details about the precursor length distribution are known, keep parameters unchanged

Class I 'combined predictor' - example

MHC-I Processing Predictions

Prediction Method Version	2013-02-22 [Older versions]
	Specify Sequence(s)
Enter protein sequence(s) in FASTA format (Browse for sequences in NCBI)	>LCMV Armstrong, Protein GP McQIVTMFEALPHIIDEVINIVIIVLIVITGIKAVYNFATCGIFALISFLLLAGRSCGM YGLKGPDIVKGYQFKSVEPDMSHLNLTMENACSANNSHHVISMGTSGLELFTFNDSII SHNFCNLTSAFNKKTFDHTLMSIVSSLHLSIRGNSNYKAVSCDFNNGITIQYNLTFSDA QSAQSQCRTFRGRVLDMFRTAFGKYMRSGMGWTGSDGKTTMCSGTSYQYLIIQNRTWE NHCTYAGPFGMSRILLSQEKTKFFTRRLAGTFTWTLSDSSGVENPGGYCLTKWMILAAE LKCFGNTAVAKCNVNHDAEFCDMLRLIDYNKAALSKFKEDVESALHLFKTTVNSLISDQ LLMRNHLRDLMGVPYCNYSKFWYLEHAKTGETSVPKCWLVTNGSYLNETHFSDQIEQEA DNMITEMLRKDYIKRQGSTFLALMDLLMFSTSAYLVSIFLHLVKIPTHRHIKGGSCPKP HRLTNKGICSCGAFKVPGVKTVWKRR
Or select file containing sequence(s)	Browse No file selected.
Choose sequence format	auto detect format
	Choose a Prediction Method
Prediction Method	IEDB recommended Help on prediction method selections
	Specify what to make binding predictions for
MHC source species	mouse
Select MHC allele(s)	Allele Length H-2-Kb 10 O
	Proteasomal cleavage prediction
Specify proteasome type	immuno 🔽
	TAP transport predictions
Maximum precursor extension	1
Alpha factor	0.2
	Specify Output
Output format	XHTML table
	Submit Reset

Class I 'combined predictor' - example

MHC-I Processing Prediction Results

http://tools.iedb.org/processing/

Input Sequences

#	Name	Sequence
1	LCMV Armstrong, Protein GP	eq:mgqivtmfealphiideviniviiviiviiviigikavynfatcgifalisfllagrs CGMYGLKGPDIYKGVYQFKSVEFDMSHLNLTMPNACSANNSHHYISMGTSGLELTF TNDSIISHNFCNLTSAFNKKTFDHTLMSIVSSLHLSIRGNSNYKAVSCDFNNGITI QYNLTFSDAQSAQSQCRTFRGRVLDMFRTAFGGKYMRSGWGWTGSDGKTTWCSQTS YQYLIIQNRTWENHCTYAGPFGMSRILLSQEKTKFFTRRLAGTFTWTLSDSSGVEN PGGYCLTKWMILAAELKCFGNTAVAKCNVNHDAEFCDMLRLIDYNKAALSKFKEDV ESALHLFKTTVNSLISDQLLMRNHLRDLMGVPYCNYSKFWYLEHAKTGETSVPKCW LVTNGSYLNETHFSDQIEQEADNMITEMLRKDYIKRQGSTPLALMDLLMFSTSAYL VSIFLHLVKIPTHRHIKGGSCPKPHRLTNKGICSCGAFKVPGVKTVWKRR
2	LCMV Armstrong, Protein NP	MSLSKEVKSFQWTQALRRELQSFTSDVKAAVIKDATNLLNGLDFSEVSNVQRIMRK EKRDDKDLQRLRSLNQTVHSLVDLKSTSKKNVLKVGRLSAEELMSLAADLEKLKAK IMRSERPQASGVYMGNLTTQQLDQRSQILQIVGMRKPQQGASGVVRVWDVKDSSLL NNQFGTMPSLTMACMAKQSQTPLNDVVQALTDLGLLYTVKYPNLNDLERLKDKHPV LGVITEQQSSINISGYNFSLGAAVKAGAALLDGGNMLESILIKPSNSEDLLKAVLG AKRKLNMFVSDQVGDRNPYENILYKVCLSGEGWPYIACRTSIVGRAWENTTIDLTS EKPAVNSPRPAPGAAGPPQVGLSYSQTMLLKDLMGGIDPNAPTWIDIEGRFNDPVE IAIFQPQNGQFIHFYREPVDQKQFKQDSKYSHGMDLADLFNAQPGLTSSVIGALPQ GMVLSCQGSDDIRKLLDSQNRKDIKLIDVEMTREASREYEDKVWDKYGWLCKMHTG IVRDKKKKEITPHCALMDCIIFESASKARLPDLKTVHNILPHDLIFRGPNVVTL

Prediction method: recommended | High Score = high efficiency Download result

Citations

Allele 🔶	# _{\$}	Start 🔶	End 🔶	Peptide Length	🔶 Peptide 🔶	Proteasome 🔶 Score	TAP Score ≑	MHC Score ¢	Processing ¢ Score	Total Score	MHC IC50[nM]
H-2-Kb	2	203	212	10	LLYTVKYPNI	1.79	0.50	-2.01	2.28	0.27	103.5
H-2-Kb	1	116	125	10	SIISHNFCNI	1.51	0.46	-1.90	1.97	0.08	78.6
H-2-Kb	2	7	16	10	VKSFQWTQAI	1.42	0.39	-2.01	1.81	-0.21	102.9
H-2-Kb	2	235	244	10	INISGYNFSI	1.53	0.41	-2.18	1.95	-0.23	149.7
H-2-Kb	1	35	44	10	VYNFATCGIE	1.42	1.27	-3.12	2.68	-0.44	1327
H-2-Kb	1	75	84	10	KSVEFDMSHI	1.50	0.47	-2.45	1.96	-0.49	283.6
H-2-Kb	1	369	378	10	YCNYSKFWYI	1.45	0.31	-2.32	1.76	-0.56	206.8
H-2-Kb	1	449	458	10	VSTELHLVKI	1.33	0.33	-2.25	1.66	-0.59	178.2

Class I 'combined predictor' - example

Allele 🔶	# ⇔	Start 🖕	End 🖕	Peptide Length	\$ P	Peptide 🔶	Proteasome 🔶 Score	TAP Score 🔶	MHC Score 🔶	Processing Score	Total Score	MHC IC50[nM] ≑
H-2-Kb	2	203	212	10	L	LLYTVKYPNL	1.79	0.50	-2.01	2.28	0.27	103.5
H-2-Kb	1	116	125	10	s	SIISHNFCNL	1.51	0.46	-1.90	1.97	0.08	78.6
H-2-Kb	2	7	16	10	v	VKSFQWTQAL	1.42	0.39	-2.01	1.81	-0.21	102.9
H-2-Kb	2	235	244	10	I	INISGYNFSL	1.53	0.41	-2.18	1.95	-0.23	149.7
H-2-Kb	1	35	44	10	v	VYNFATCGIF	1.42	1.27	-3.12	2.68	-0.44	1327
H-2-Kb	1	75	84	10	K	KSVEFDMSHL	1.50	0.47	-2.45	1.96	-0.49	283.6
H-2-Kb	1	369	378	10	Y	YCNYSKFWYL	1.45	0.31	-2.32	1.76	-0.56	206.8
H-2-Kb	1	449	458	10	v	VSIFLHLVKI	1.33	0.33	-2.25	1.66	-0.59	178.2

- Higher scores = higher efficiency for MHC-I presentation
- MHC binding score = -log10(IC50) (sign change)
- Combined scores are additive
 - Processing = proteasome + TAP
 - Total = proteasome + TAP + MHC
- Different variance in scores reflects different selectivity

Caveats / performance of processing predictions

- Co-evolution of MHC molecules to bind peptides with motifs that are generated by proteasome and TAP means that most high-affinity MHC binders are also efficiently processed
- If resources require limiting the number of peptides considered, use total score of processing predictions as additional filter
- Most importantly, step-wise processing predictions help understand *why* a given peptide may not be a good MHC ligand

<u>Recommended Alternative</u>: Use predictors directly trained on eluted ligand data



- Mass spectrometry of eluted ligands allows for the identification of a very large number of ligands in a single experiments
- Ligand sequences contain signals from both binding and processing
- NetMHCPan EL predictions (trained on eluted ligands) performs excellent, and can be used just like a regular MHC binding prediction

Incorporating Antigen Expression: Axel-F

 Increased expression of an antigen in a cell increases the likelihood that peptides derived from it are processed and presented

iScience



Volume 25, Issue 2, 18 February 2022, 103850

Article

Combined assessment of MHC binding and antigen abundance improves T cell epitope predictions

Zeynep Koşaloğlu-Yalçın ¹, Jenny Lee ¹, Jason Greenbaum ¹, Stephen P. Schoenberger ^{2, 3}, Aaron Miller ^{2, 3}, Young J. Kim ⁴, Alessandro Sette ^{1, 5}, Morten Nielsen ^{6, 7}, Bjoern Peters ^{1, 5, 8} \Re 😂

 Axel-F tool integrates expression data into MHC ligand predictions



IEDB Analysis Resource - Labs

Home Help Example Reference Download Contact

Axel-F

Antigen eXpression based Epitope Likelihood-Function

	Specify Sequence(s)
Upload CSV or FASTA file. (Make sure CSV file contains header.)	Upload CSV file here allele,peptide,tpm HLA-A*01:01,MLEDSDEHLDY,4.55 HLA-B*37:01,GEFSEEAKF,16.72 HLA-C*15:02,SSMFSPLKM,4.27 HLA-B*07:02,QRPILTIITL,37.13 HLA-B*35:01,VPEDDGTVSA,9.28 HLA-A*29:02,ITSPVDVVKTRY,52.03 HLA-A*29:02,HLVGYGGRY,23.66
Allele Selection	From CSV Select Allele
Select TPM source	From CSV O TCGA O Manual Input
	Submit Reset

Class I Processing + immunogenicity tools available in the IEDB <u>http://tools.iedb.org/main/tcell/</u>

IEDB Analysis Resource

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Overview T	Cell Tools	B Cell Tools	Analysis Tools	Tools-API	Usage	Download	Datasets	Contribute Too	ls F	References		
T Cell Epi	topes -	Processin	g Prediction									
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	nitonoo	Processin	- Dradiation							
Cell E	ols predict epi	itope candida	tes based upon	the proces	sing of p	eptides in th	ne cell.			
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Class I immunogenicity prediction

- Approach: Assemble two datasets of peptides with similar MHC binding affinity, that are (i) recognized or (ii) not recognized by T cells
- Enrichment of W,F,I and depletion of S,M,K in immunogenic peptides
- Use enrichments to calculate propensity scores



Class I immunogenicity prediction -example

http://tools.iedb.org/immunogenicity/

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Class I Immun	ogenicity	
	Specify sequence(s) *	
Enter peptide sequence(s) (Browse for sequences in NCBI)	FIAGLIAIV LITGRLQSL RLNEVAKNL KAVYNFATC FQPQNGQFI	
Or select file containing sequence(s)	Browse No file selected.	
	Choose which positions to mask	
Specify which positions to mask	Default (1st, 2nd, and C-terminus amino acids) Custom User Defined (Comma separated numbers) Peptide lengths must be equal when using custom masking.	Mask positions that are MHC anchors
	Submit Reset	
*The tool was only validat	ted for 9-mer peptides. However, predictions can be made for peptides of any length.	

Class I immunogenicity prediction -example

http://tools.iedb.org/immunogenicity/

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Class I Immunogenicity Masking: default Masked variables: [1, 2, 'cterm']											
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- Scores are sums of propensity scores at all unmasked positions
- High scores = peptide is more likely to be immunogenic

Class I immunogenicity prediction caveats / performance

- Experimentally, many MHC binding peptides can be immunogenic (~50%)
- Cross validation gave AUC values ~ 0.65. Test on independent blind set gave AUC = 0.69
- Recommendation: Use as filter (cutoff 0) if high specificity is desired. Suggested cutoff is 0



Class I Summary

- Processing predictions give insights into which steps in the antigen processing pathway supports / hinders presentation of a peptide
- NetMHCPan 4.0 EL scores, which are trained on both MHC binding and ligand elution data are a straightforward replacement of MHC binding
- Integration of expression data of antigens via Axel-F further improves ligand prediction (if such data is available)
- Immunogenicity predictions can provide a further filter of more immunogenic peptides

CD4 T cell epitopes (MHC class II)



Class II Processing + immunogenicity tools available in the IEDB <u>http://tools.iedb.org/main/tcell/</u>

IEDB Analysis Resource

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Overview	T Cell Tools	B Cell Tools	Analysis Tools	Tools-API	Usage	Download	Datasets	Contribute Tools	References	
	Enitones -	Processin	a Prediction							
hese to	ols predict e	pitope candida	ates based upor	the proces	sing of p	eptides in tl	ne cell.			
Protease	omal cleavag	e/TAP transp	ort/MHC class I	combined	predictor					
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MHC-NF	P: Prediction	of peptides na	aturally process	ed by the N	<u>/IHC</u>					
MHO	C-NP employ	s data obtain	ed from MHC e	elution exp	eriments	in order to	assess th	e probability that	at a given pe	eptide is naturally
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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/



MHCII-NP -example

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

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MHC II NP results

Top 5 peptides per protein:

	Seq name	Peptide start	Peptide end	Peptide length	Peptide	N motif	C motif	Cleavage probability score	Cleavage probability percentile rank		
1	SPIP15848IARSB_HUMAN ARYLSULFATASE B OS=HOMO S	510	524	15	VPVYFPAQDPRCDPK	SVP	PKA	1.75814	0.00		
2	SPIP15848IARSB_HUMAN ARYLSULFATASE B OS=HOMO S	2	16	15	GPRGAASLPRGPGPR	MGP	PRR	1.73735	0.02		
3	SPIP15848IARSB_HUMAN ARYLSULFATASE B OS=HOMO S	247	261	15	VPEEYLKPYDFIQDK	QVP	DKN	1.48840	0.04		
4	SPIP15848IARSB_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		
Cor	omplete results										

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).

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

Genome Medicine

RESEARCH

Footprints of antigen processing boost MHC class II natural ligand predictions

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



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.

 $\underline{\mathsf{Paul S}^1}, \underline{\mathsf{Karosiene E}^1}, \underline{\mathsf{Dhanda SK}^1}, \underline{\mathsf{Jurtz V}^2}, \underline{\mathsf{Edwards L}^1}, \underline{\mathsf{Nielsen M}^{2,3}}, \underline{\mathsf{Sette A}^{1,4}}, \underline{\mathsf{Peters B}^{1,4}}.$

PMID: 30127785 PMCID: PMC6087742 DOI: 10.3389/fimmu.2018.01795

Class II Processing + immunogenicity tools available in the IEDB <u>http://tools.iedb.org/main/tcell/</u>

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Overview T Cell Tool	B Cell Tools	Analysis Tools	Tools-API	Usage	Download	Datasets	Contribute Tool	s References	
T Cell Enitones	- Processing	Prediction							
These tools predict	epitope candida	tes based upon	the process	sing of pe	eptides in tl	ne cell.			
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This tool comb peptide's intrir	ines predictors sic potential of	of proteasoma being a T cell	al processin epitope.	g, TAP t	ransport, a	and MHC b	inding to prod	uce an overal	l score for each
Neural network bas	ed prediction of	proteasomal c	leavage site	s (NetCl	nop) and T	cell epitop	es (NetCTL an	d NetCTLpan)
NetChop is a p epitopes along	redictor of prot a protein sequ	easomal proce ence. It also e	essing base mploys a n	d upon a eural ne	i neural ne twork arch	twork. Ne itecture.	tCTL and NetC	TLpan are pro	edictors of T cell
MHC-NP: Prediction	n of peptides na	turally process	ed by the M	<u>HC</u>					
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Cell Epitopes	- Immunoge	nicity Predic	ction	to elicit a	an immune	response.	<u> </u>		
F cell class I pMHC	immunogenicity	y predictor							
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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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Home Help Example	Reference Contact								
	Specify Sequence(s)								
Enter epitope sequence(s) in PLAIN or FASTA format	>sp P01588 EP0_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1 MGVHECPAWLWLLLSLLSLPLGLPVLGAPPRLICDSRVLERYLLEAKEAENITTGCAEHC SLNENITVPDTKVNFYAWKRMEVGQQAVEVWQGLALLSEAVLRGQALLVNSSQPWEPLQL HVDKAVSGLRSLTTLLRALGAQKEAISPPDAASAAPLRTITADTFRKLFRVYSNFLRGKL KLYTGEACRTGDR								
Or upload epitope sequence(s) from a file	Browse No file selected.								
	Choose a prediction method								
Prediction method:	IEDB recommended (combined)								
	Specify Output 7-allele								
Sort Peptides by:	Position in Protein Score/Percentile Rank Immunogencity								
Select maximum percentile rank threshold:	50 Position in Protein								
Enter the Job Name (Optional)									
Email address (optional)									
	Submit Reset								

Class II immunogenicity prediction - example

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

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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	Protein Description	Peptide	Start	End	Combined	Immunogenicity	Peptide core	Median	HLA-						
Number	*	•	٠	•	Score	Score	•	Rank (7- allele)	DRB1:03:01	DRBT:07:01	DRBT:15:01	DRB5:01:01	DRB5:02:02	DKB4:01:01	DRB5:01:01
(1)	spIP01588 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	spJP01588JEPO_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	spIP01588IEPO_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	spIP01588IEPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1	VSGLRSLTTLLRALG	126	140	44.95964	86.8991	LTTLLRALG	17.0	12.0	17.0	9.3	70.0	20.0	20.0	1.3
1	spIP01588IEPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1	SLTTLLRALGAQKEA	131	145	42.78744	69.4686	LLRALGAQK	25.0	47.0	46.0	21.0	89.0	25.0	14.0	1.6
1	splP01588IEPO HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1	PLRTITADTFRKLFR	156	170	46.18064	85.4516	LRTITADTF	20.0	6.1	47.0	40.0	8.0	20.0	20.0	20.0
1	spIP01588IEPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1	TADTFRKLFRVYSNF	161	175	46.66984	44.6746	FRKLFRVYS	48.0	63.0	58.0	23.0	48.0	33.0	53.0	24.0
1	splP01588 EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1	RKLFRVYSNFLRGKL	166	180	13.8966	22.4415	FRVYSNFLR	8.2	53.0	8.2	0.12	27.0	4.8	26.0	1.5

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).

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



Predicting HLA CD4 Immunogenicity in Human Populations.

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Class II Summary

- Similar to MHC class I, enhancement of epitope prediction efficacy is minor compared to using MHC binding predictions alone
- Prediction of naturally eluted ligands is greatly improved with processing predictions
- As of now, recommendation is to stick to allele specific MHC binding predictions (NetMHCPanII), or the 7-allele method for broad populations

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