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## Contriving a novel multi-epitope subunit vaccine from *Plasmodium falciparum* vaccine candidates against malaria

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## SUPPLEMENTARY MATERIAL

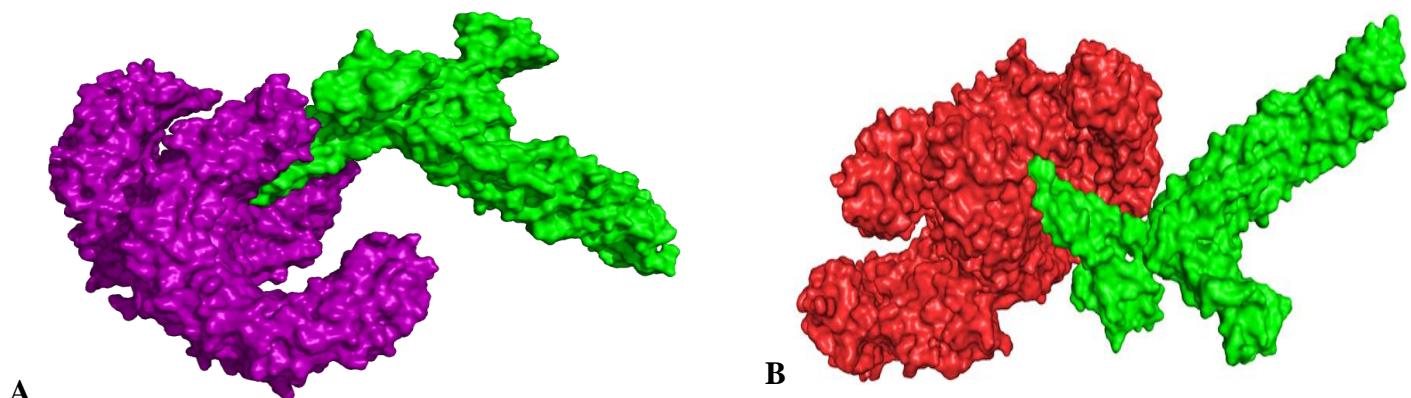
**Table S1.** Conformational B-cell epitopes

Epitope	Residues	No. of Residues	Score
1	A:E124, A:N125, A:N126, A:E127, A:N128, A:V129, A:G130, A:P131, A:G132, A:P133, A:G134, A:A135, A:K136, A:N137, A:F138, A:Y139, A:N140, A:I141, A:S142, A:N143, A:E144, A:N145, A:G146, A:D147, A:N148, A:T149, A:G150, A:P151, A:G152, A:P153, A:G154, A:D155, A:N156, A:T157, A:F158, A:N159, A:N160, A:N161, A:N162, A:N163, A:N164, A:M165, A:D166, A:N167, A:K168, A:K169, A:G170, A:P171, A:G172, A:P173, A:G174, A:L175, A:P176, A:S177	54	0.758
2	A:K273, A:D274, A:N275, A:N278, A:N281, A:D282, A:I284, A:N285, A:N286, A:D287, A:K288, A:K289, A:N290, A:K291, A:E292, A:N293, A:M294, A:N295, A:N296, A:E297, A:K298, A:N299, A:D300, A:N301, A:K302, A:D303, A:N304, A:K305, A:K306, A:K307, A:K308, A:I309, A:T310, A:Y311, A:D312, A:K313, A:Y314, A:N315, A:K316, A:N317, A:K318, A:E319, A:N320, A:M321, A:N322, A:N323, A:K324, A:D403, A:K404, A:K406, A:D407, A:V408, A:S409, A:L410, A:V411, A:V412, A:E413, A:K414, A:K415, A:T416, A:V417, A:E418, A:I419, A:S420, A:G421, A:E422, A:S423, A:L424, A:E425, A:N426, A:N427, A:E428, A:M429, A:D430, A:K431, A:H432, A:H433, A:H434, A:H435	79	0.742
3	A:A1, A:P2, A:P3, A:H4, A:A5, A:L6, A:S7, A:E8, A:A9, 81 0.73, A:A10, A:A11, A:K12, A:I13, A:I14, A:S15, A:G16, A:D17, A:A18, A:I20, A:K21, A:A22, A:Y24, A:K25, A:V26, A:R27, A:F28, A:D29, A:I30, A:K31, A:D32, A:K33, A:A34, A:A35, A:Y36, A:R37, A:K51, A:D52, A:L53, A:E54, A:A55, A:D56, A:I57, A:A58, A:A59, A:Y60, A:Y61, A:V62, A:D63, A:V64, A:D65, A:V66, A:P67, A:A68, A:M69, A:A70, A:A71, A:Y72, A:E73, A:E74, A:H75, A:D76, A:I77, A:T78, A:T79, A:T80, A:L81, A:A82, A:A83, A:Y84, A:R85, A:S86, A:I87, A:E88, A:T89, A:S90, A:I91, A:V92, A:I93, A:A94, A:A95, A:Y96	81	0.73
4	A:D249, A:G250, A:P251	3	0.512

**Table S2.** Physicochemical properties of vaccine construct

Properties	Value/Score
<b>Number of amino acids</b>	437
<b>Molecular weight</b>	49208.25
<b>Theoretical pI</b>	5.99
<b>Formula:</b>	C <sub>2127</sub> H <sub>3374</sub> N <sub>616</sub> O <sub>715</sub> S <sub>6</sub>
<b>Total number of atoms</b>	6838
<b>The estimated half-life</b>	4.4 hours (mammalian reticulocytes, <i>in vitro</i> ). >20 hours (yeast, <i>in vivo</i> ). >10 hours ( <i>Escherichia coli</i> , <i>in vivo</i> ).
<b>Instability index</b>	The instability index (II) is computed to be 39.36. This classifies the protein as <b>stable</b>
<b>Aliphatic index</b>	61.40
<b>GRAVY</b>	-1.294
<b>Antigenicity</b>	0.7515
<b>Allergenicity</b>	Probable non-allergen

GRAVY: Grand average of hydropathicity



**Figure S1.** The designed vaccine (green) interaction pattern with (A) TLR2 (purple) and (B) TLR4 (red).

**Table S3. B-cell epitopes screening**

Rank	Sequence	Start position	Score	Antigenicity	allergenicity	toxicity	conservancy
<b>LSA 3-C (V750-K1433)</b>							
1	DEDIDEDIEEDVEEDI	556	0.94	+	+	-	+
2	DEDIDEDIDEDEDIGEDK	580	0.93	+	-	-	+
3	KGSILDMLKGDMELGD	453	0.92	-	-	-	-
3	VEHIISGDAHKGLEE	428	0.92	+	-	-	-
4	TVSIIEMEENIVDVL	165	0.91	-	+	-	-
5	TESIKDKEKDVSLLVVE	209	0.90	+	-	-	-
6	QETVTTEHVEQNRYVVDV	95	0.89	+	+	-	-
6	TVEISGESLENNEMDK	8	0.89	+	-	-	-
6	RFDIKDKEPKDEIVEV	537	0.89	+	-	-	-
6	EHDITTLDEVVELKD	312	0.89	+	-	-	-
7	EVEEEKKLEEVHELKE	411	0.88	-	-	-	-
8	DKEVSKALESKNDVTN	649	0.87	+	-	-	+
8	EEDVEEDIEEDKVEDI	564	0.87	+	+	-	-
9	ETSIVIQSEEKVSDLNE	49	0.86	+	-	-	
10	SGLKKHVDEVMKYVQK	628	0.85	-	-	-	-
11	DEVIDLIVQKEKRIEK	596	0.84	-	-	-	-
11	KKITKKVRFDIKDK	529	0.84	+	+	-	-
11	MDEEQMKTRKKAQRPK	498	0.84	+	-	-	-
12	DKMIDAVEESIEISSD	189	0.82	+	-	-	-
13	ALSEDSKEIIDAKDDT	289	0.81	+	-	-	-
14	DEDIGEDKDEVIDLIV	588	0.80	+	-	-	-
14	LKTIETDILEEKKEIE	367	0.80	-	+	-	-
14	IQENLLTGMRFSIETS	36	0.80	+	-	-	-
15	DMELGDMKDKESEDVT	463	0.78	-	-	-	-
15	KGLEEDDLEEVDDLKG	439	0.78	-	-	-	-
15	SKLIEETQELNEVEAD	260	0.78	+	-	-	-
16	ALESKNDVTNLKQHQ	655	0.77	+	-	-	-
16	FSEIFDNVKGIQENLL	26	0.77	-	+	-	-
16	HVEQNVYVDVDVPAMK	101	0.77	+	+	-	-
17	KDVEEDKIEKVSDLKD	326	0.76	+	-	-	-
17	SDVITVEEIKDEPVQK	144	0.76	-	-	-	-
18	KELESEILEDYKELKT	354	0.75	+	-	-	-
18	EELMKDAVEINDITSK	246	0.75	-	-	-	-
19	NVLKQNQDFSKVKNF	664	0.74	-	-	-	-
19	EEKEDLTDKMDIAVEE	182	0.74	-	+	-	-

19	VDVPAMKDQFLGILNE	110	0.74	+	-	-	-	-
20	VSSILDNIENMKEGLL	67	0.73	+	-	-	-	-
20	LEDVTAKLGERVERESLK	474	0.73	+	-	-	-	-
20	AEEIKDLEADILKEVS	393	0.73	+	-	-	-	-
20	KEIIDAKDDTLEKVIE	295	0.73	-	+	-	-	-
20	VVEEVQDNDMDESVEK	222	0.73	+	-	-	-	-
20	EEIKDEPVQKEVEKET	150	0.73	+	-	-	-	-
21	RKKAQRPKLEEVLLKE	506	0.72	+	-	-	-	-
21	EIEKDHFKEKFEAAE	380	0.72	-	+	-	-	-
21	TLEKVIEEEHDITTTL	304	0.72	-	-	-	-	-
22	EKVSDDLKDLEEDILKE	334	0.71	+	-	-	-	-
22	EENIVDVLEEEKEDLT	173	0.71	+	-	-	-	-
23	DEVMKYVQKIDKEVDK	635	0.70	-	-	-	-	-
23	KEKRIEKVKEKKKKLE	605	0.70	+	+	-	-	-
24	NKLENISSTEGVQETV	83	0.69	-	-	-	-	-
24	LEEVDLKGSIIDMLK	446	0.69	-	-	-	-	-
24	EEDILKEVKEIKELES	343	0.69	-	+	-	-	-
25	KVLELKNMEEEELMKDA	237	0.68	+	+	-	-	-
26	PKLEEVLLKEEVKEEP	512	0.67	+	-	-	-	-
27	NEVEADLIKDMEKLKE	270	0.65	-	+	-	-	-
28	KKLEKKVEEGVSGLKK	617	0.63	+	-	-	-	-
29	LDKVEETVEISGESLE	2	0.61	+	+	-	-	-
30	VQKIDKEVDKEVSKAL	641	0.59	-	-	-	-	-
31	NIENMKEGLLNKLENI	73	0.57	-	-	-	-	-
32	MDESVEKVLELKNMEE	231	0.55	-	-	-	-	-
33	LNEAGGLKEMFFNLED	123	0.54	-	-	-	-	-
34	LKEVSSLEVEEEKKLE	404	0.53	-	-	-	-	-
35	QSEEKVDLNENVVSSI	55	0.52	+	-	-	-	-

#### MSA-Tr4 (A805-P1093)

1	TITINNGTTSNTIENN	207	0.94	-	-	-	-	-
2	ISNENGDNTFNNNNNN	7	0.93	-	+	-	-	-
3	HKDNDSRYTDNSNKNR	35	0.91	+	-	-	-	-
4	DNKKRMYNYNKHKDND	24	0.89	+	+	-	-	-
4	NKEAENSNTEQNDNNN	226	0.89	+	+	-	-	-
5	TSDILYKDIEENKNTE	185	0.88	+	-	-	-	-
6	SKVTGDSVENINEQTN	269	0.85	+	-	-	-	-
6	NTEQNDNNNNNDNNNNI	233	0.85	+	-	-	-	+
7	DVLLIETITINNGTTS	201	0.84	-	-	-	-	-
7	HHTNVYEPNDEEKQNE	160	0.84	+	-	-	-	-
8	YNRYKDNNYYYYNSDN	74	0.83	+	+	-	-	-
9	RKRYIRKKTYNKLSYF	95	0.82	+	-	-	-	-
10	SNTIENNKKDSNKEAEN	216	0.81	+	-	-	-	-

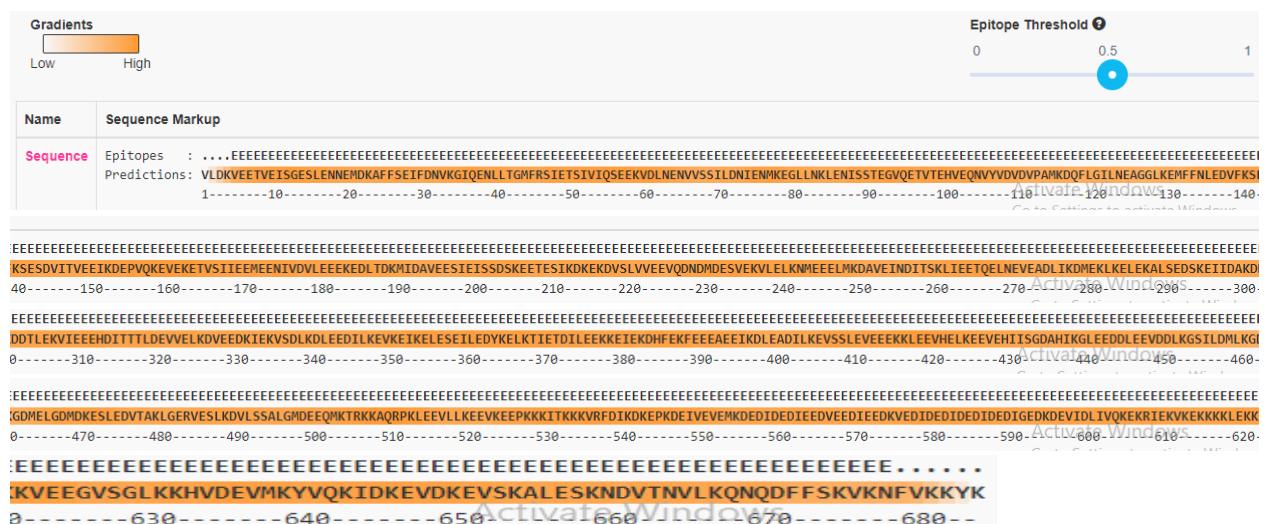
11	KDIEENKNTEVDLLIE	191	0.74	+	+	-	-
11	SFPLNKPQDHEAFYNL	142	0.74	-	-	-	-
12	DNKEEDMNENNNSKVN	256	0.73	+	-	-	-
12	DNELPEQTESFPLNKP	133	0.73	-	-	-	-
12	IKGNSEEFSFDNELPE	123	0.73	+	-	-	-
13	NKNRDNYNRNPKDNNT	54	0.72	+	-	-	-
14	YTDNSNKNRDNDSNKNR	42	0.71	+	+	-	-
15	DKNNNTNRDNYNRYKDN	65	0.69	+	-	-	-
16	NLPSLKSIIYNKIKGN	111	0.64	+	-	-	-
17	PNDEEKQNEQKLKDQI	167	0.63	+	-	-	-
18	NNYYNNSDNNNYNER	80	0.53	+	-	-	-

#### MSP10 R1 (D29-N188)

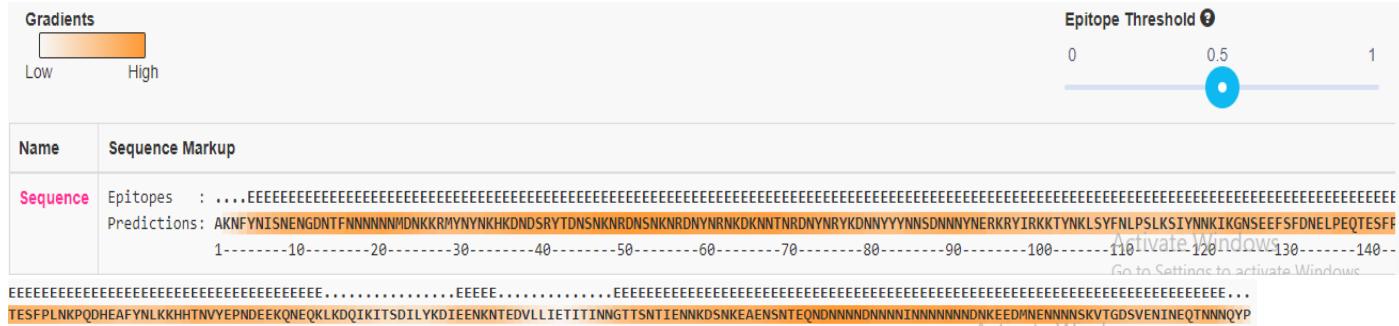
1	KNTSQKKITYDKYNKN	4	0.93	+	+	-	-
2	KITYDKYKNKRNKMN	10	0.87	+	-	-	-
3	NKENMNNEKNDNKDNK	19	0.86	+	-	-	-
4	NKDNKDNLYNDNINND	30	0.85	+	-	-	-
5	SMKHYKDSLNSKLNNE	62	0.84	+	-	-	-
6	NEDEYKF LSMKHYKDS	54	0.80	+	-	+	-
7	NVENNENIENNENNEN	107	0.71	+	-	-	-
8	TQGSQHFNENIENNEN	92	0.69	+	-	-	-
9	NIENIENNENNENNEN	122	0.67	+	-	-	-
10	ENDHMNYLIRKRKDNT	77	0.66	+	+	-	-
10	NNENIENNENNENNEN	134	0.66	+	-	-	+
11	NNENNENNENSSIMNS	140	0.63	+	+	-	-
11	ENIENNENVENNENIE	100	0.63	+	-	-	-
12	DNINNDNINNDNINNE	40	0.62	+	+	-	-

Highlighted epitopes in red colour were selected

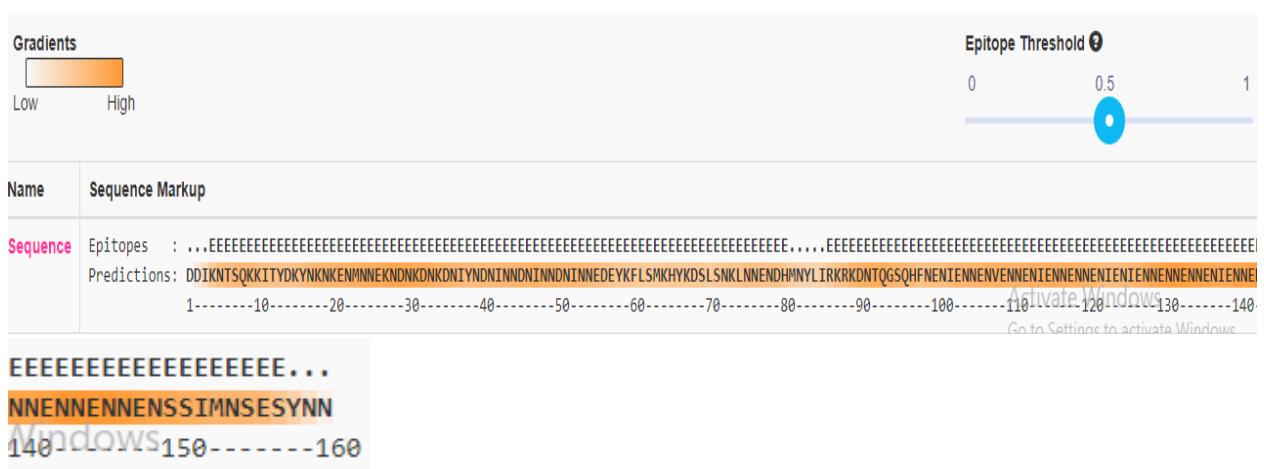
- a) BepiPred-2.0: Sequential B-Cell Epitope Predictor (<https://services.healthtech.dtu.dk/cgi-bin/webface2.cgi?jobid=644687DE0000029F864CC222&wait=20>)



- b) BepiPred-2.0: Sequential B-Cell Epitope Predictor (<https://services.healthtech.dtu.dk/cgi-bin/webface2.cgi?jobid=64467F25000046E485452671&wait=20>)



- c) BepiPred-2.0: Sequential B-Cell Epitope Predictor (<https://services.healthtech.dtu.dk/cgi-bin/webface2.cgi?jobid=64467F36000049882ECA620A&wait=20>)



**Figure S2.** B-cell and CD4<sup>+</sup> epitopes screening output from BepiPred-2.0 server for (a) LSA 3-C, (b) MSA-Tr4 and (c) MSP10 R1

**Table S4: CD4+ epitopes screening**

PEPTIDE	EPITOPES	HLA-II ALLELES
<b>LSA 3-C (V750-K1433)</b>		
		<a href="https://services.healthtech.dtu.dk/cgi-bin/webface2.cgi?jobid=6446B171000067F96562F4EB&amp;wait=20">https://services.healthtech.dtu.dk/cgi-bin/webface2.cgi?jobid=6446B171000067F96562F4EB&amp;wait=20</a>
	ILEDYKELKTIETDI	DRB1_0101, DRB1_0401
	VKEIKELESEILEDY	DRB1_0101, DRB1_1201, DRB1_1501
	ESEILEDYKELKTIE	DRB1_0301
	KKKVRFDIKDKEPKD	DRB1_0301, DRB1_1302, DRB4_0101
	SIEISSSDSKEETESI	DRB1_0301
	EKALSEDSKEIIDAK	DRB1_0301
	EQNVYVDVDVPAMKD	DRB1_0301, DRB1_0401, DRB1_0405, DRB3_0101
	EHIISGDAHIKGLEE	DRB1_0301
	QNVYVDVDVPAMKDQ	DRB1_0301
	EEDIEEDKVEDIDED	DRB1_0301, DRB3_0101,
	KDQFLGILNEAGGLK	DRB1_0401, DRB1_0405
	EDVFKSESDVITVEE	DRB1_0401
	EMFFNLEDVFKSESD	DRB1_0401
	EDKIEKVSDLKDLEE	DRB1_0405
	EESIEISSSDSKEETE	DRB1_0405
	TLDEVVELKDVEEDK	DRB1_0405
	KDQFLGILNEAGGLK	DRB1_0405
	ADILKEVSSLEVEEE	DRB1_0701
	AVEINDITSKLIEET	DRB1_0701, DRB1_1501
	TEGVQETVTEHVEQN	DRB1_0701
	MEKLKELEKALSEDS	DRB1_0802, DRB1_1101
	NQDFFSKVKNFVKKY	DRB1_0802, DRB1_1302
	TGMFRSIETSIVIQS	DRB1_0901
	MEKLKELEKALSEDS	DRB1_1101
	ETSIVIQSEEKVDLN	DRB1_1201, DRB4_0101
	AEEIKDLEADILKEV	DRB1_1201, DRB4_0101
	VIDLIVQKEKRIEKV	DRB1_1201
	ELKDVEEDKIEKVSD	DRB3_0101
	SEEKVDLNENVVSSI	DRB3_0202
	YKELKTIETDILEEK	DRB4_0101
	ETSIVIQSEEKVDLN	DRB4_0101
	VEEIKDEPVQKEVEK	DRB4_0101
	AEEIKDLEADILKEV	DRB4_0101
	GESLENNEMDKAFFS	DRB4_0101
	SKEIIDAKDDTLEKV	DRB4_0101
	QDFFSKVKNFVKKYK	DRB5_0101
<b>MSA-Tr4</b>		

<b>(A805-P1093)</b>		
	KLSYFNLPSLKS1YN	DRB1_0101, DRB1_0401
	<b>AKNFYNISNENGDNT</b>	DRB1_0401, DRB1_0405, DRB1_0802
	<b>DNTFNNNNNNMDNKK</b>	DRB1_0401, DRB1_0405, DRB3_0202
	SVENINEQTNNNQYP	DRB1_0401, DRB4_0101
	DNNYYYNNSDNNNYN	DRB1_0401, DRB3_0202
	NNYYYNNSDNNNYNE	DRB1_0401
	NNINNNNNNNNDNKE	DRB1_0405, DRB3_0202
	HEAFYNLKKHHTNVY	DRB1_0802, DRB1_1101
	LKDQIKITSDILYKD	DRB1_1201, DRB1_1302
	<b>LPSLKSIYNNKIKGN</b>	DRB1_1201, DRB1_1302, DRB1_1501
	IETITINNGTTSNTI	DRB1_1302, DRB3_0202
	FYNLKKHHTNVYEPN	DRB1_1302
	SEEFSFDNELPEQTE	DRB3_0101
	NSKVTDGSVENINEQ	DRB3_0101
	RDNYNRNKDKNNTNR	DRB3_0202
	YYYYNNSDNNNYNER	DRB3_0202
<b>MSP10 R1 (D29-N188)</b>		
	<b>NENIENIENNEN</b>	DRB1_0401, DRB1_0405, DRB4_0101
	KDNIYNDNINNDNIN	DRB1_0405
	MKHYKDSLSNKLNN	DRB1_0701, DRB1_0901
	EYKFLSMKHYKDSL	DRB1_0802
	EDEYKFLSMKHYKDS	DRB1_1201
	QKKITYDKYNKNKEN	DRB1_1302
	NENIENNENVENNEN	DRB3_0202
	YDKYNKNKENMNNEK	DRB3_0202
	NENIENNENNEN	DRB3_0202
	IENIENNENNEN	DRB3_0202
	NENIENNENNENIEN	DRB3_0202
	DNIYNDNINNDNINN	DRB3_0202
	NENVENNENIENNEN	DRB3_0202

Highlighted epitopes in red colour were selected