



Supplemental Figure 1. Development and optimization of an HLA DPB1*0101 (DPw1) binding assay

Peptide 3172.0005 (sequence EKKYFAATQFEPLAA) was utilized as the radioligand in direct binding assays for DPB1*0101, as described in the binding methods. Optimum pH and incubation temperature was examined (Figure A), to include pH range from 5 to 8, and at room temp and 37C. With both temperature conditions pH 5.5 was shown to be the optimal pH used. Optimum length of capture was also examined (Figure B) in 24 hour increments from 48 hours to 96 hours time period. Due to the evaporation issues along with higher background noise found in incubating an assay at 37C both room temperature and 37C were examined simultaneously with the differing incubation time periods (48-96 hours). An optimum binding assay should have absolute counts of at least three times higher than the background counts. Though the absolute counts for incubations at 37C are higher, figure C illustrates the greater S/N ratio (absolute counts/background counts) of assays incubated at room temperature. In this case, 72 hours at room temp with pH of 5.5 was preferred due to the higher background noise found at 37C. Antibody Specificity was determined by antibody capture using B7/21 (anti DP), HB180 (pan Class 2), LB3.1 (anti DR), and SPVL3 (anti DQ) (figure D). As shown in figure D, B7/21 antibody (anti DP) exhibits the highest amount of binding with the DPB*0101 assay with the expected HB180 (pan class II) also showing binding although significantly less.

Supplemental Table 1. Single amino acid substitution analysis of the binding specificity of HLA DPB1*0401 and DPB1*0402.

A. HLA DPB1*0401¹

Substitution	E 1	K 2	K 3	Y 4	F 5	A 6	A 7	T 8	Q 9	F 10	E 11	P 12	L 13	A 14	A 15
A	0.92	0.44	0.49	0.20	0.040	1.0	1.0	2.1	1.4	0.020	0.87	8.9	0.59	1.0	1.0
C	0.46	0.24	0.32	0.37	0.043	0.38	0.60	0.35	1.5	0.025	1.1	2.9	0.15	3.3	2.5
D	1.2	0.27	0.24	0.94	0.077	0.38	0.72	0.39	0.76	0.0034	0.28	1.1	0.29	2.2	0.52
E	1.0	0.13	0.26	0.28	0.0019	0.78	0.85	1.4	1.2	0.0028	1.0	0.82	0.56	1.7	1.7
F	0.43	0.40	5.4	0.41	1.0	2.1	2.3	0.57	3.4	1.0	4.1	1.1	0.63	1.9	0.99
G	1.4	0.45	0.52	0.25	0.012	0.33	1.5	3.2	2.3	0.056	1.1	1.4	0.11	1.2	1.8
H	0.82	0.26	0.39	0.34	0.078	0.86	1.0	0.98	1.0	0.0083	1.8	1.0	0.058	2.6	0.91
I	0.74	0.15	0.91	0.57	0.28	0.91	2.4	0.85	2.2	0.10	1.2	0.88	0.62	3.2	0.48
K	1.1	1.0	1.0	0.31	0.0060	1.4	0.75	0.17	4.6	0.0021	0.73	0.71	0.032	2.1	0.13
L	0.79	0.37	1.2	0.60	0.18	1.4	0.99	111	0.90	0.20	2.7	78	1.0	2.4	1.3
M	1.0	0.23	1.6	0.67	0.20	1.4	1.5	3.0	1.9	0.41	1.6	37	0.30	0.55	0.032
N	0.44	0.16	0.12	0.58	0.0041	0.79	1.2	0.38	1.9	0.0043	0.23	3.9	0.39	1.3	0.31
P	0.65	0.27	0.23	1.2	0.010	0.049	0.72	1.3	1.4	0.0073	1.6	1.0	0.21	2.4	0.57
Q	0.68	0.40	0.39	0.92	0.015	1.8	2.3	2.7	1.0	0.029	1.7	5.0	0.20	1.7	1.0
R	0.89	0.30	0.082	0.34	0.016	2.1	1.5	0.22	2.1	0.0015	1.0	1.7	0.15	3.4	0.16
S	0.88	0.28	0.81	1.4	0.0063	2.3	3.6	1.3	4.7	0.015	2.9	3.2	0.48	2.1	0.30
T	4.0	0.22	0.53	1.1	0.0031	1.4	2.7	1.0	1.1	0.0016	1.0	4.4	0.58	2.0	0.14
V	0.61	0.63	1.0	0.45	0.41	2.7	1.1	3.0	0.89	0.019	66	1.2	2.3	1.6	0.41
W	0.44	0.24	1.6	0.56	0.26	2.0	1.8	0.18	3.0	0.59	2.7	1.6	1.2	1.1	1.6
Y	0.93	0.23	3.9	1.0	0.36	1.5	4.2	0.29	44	1.3	2.2	2.5	0.73	2.0	0.70
Summary²:															
Geomean	0.83	0.30	0.61	0.54	0.040	0.98	1.4	1.0	2.0	0.026	1.5	2.6	0.34	1.8	0.55
SF	0.70	2.0	0.96	1.1	15	0.60	0.42	0.56	0.29	23	0.38	0.23	1.7	0.32	1.1
10-fold effects	0	0	1	0	13	1	0	1	1	14	1	2	2	0	1

1. Values reflect the relative binding capacity of a peptide bearing the indicated residue substituted in the specified position of peptide EKKYFAATQFEPLAA).

2. Geomean: geometric mean relative binding capacity of all substitutions at the indicated position. SF (specificity factor): ratio of the geomean of all substitutions at the specified position to the geomean of all 300 possible substitutions. 10-fold effects: number of substitutions at each position with a 10-fold increase or decrease in binding capacity. Bold font highlights geomean <0.25, SF >2.4 or ≥7 10-fold effects on binding.

B. HLA DPB1*0402

Substitution	E 1	K 2	K 3	Y 4	F 5	A 6	A 7	T 8	Q 9	F 10	E 11	P 12	L 13	A 14	A 15
A	1.4	0.57	0.60	0.27	0.030	1.0	1.0	1.6	6.4	0.075	0.54	3.6	0.38	1.0	1.0
C	1.1	1.2	0.89	0.98	0.11	0.59	0.79	0.55	1.9	0.16	0.77	0.70	0.051	1.6	1.4
D	0.39	0.59	0.83	0.47	0.022	0.58	2.0	0.44	5.3	0.0075	0.39	0.52	0.30	0.73	1.5
E	1.0	0.45	1.5	0.35	0.015	0.65	1.1	1.1	2.0	0.0090	1.0	2.2	0.23	1.5	0.75
F	0.12	0.85	0.77	0.51	1.0	2.4	3.4	0.86	3.0	1.0	1.7	2.2	0.36	1.5	2.7
G	3.0	0.91	0.48	0.30	0.015	1.2	3.5	1.1	1.6	0.054	0.41	1.6	0.12	1.1	1.7
H	1.8	0.19	0.43	0.23	0.14	0.92	1.4	0.74	0.75	0.0069	0.47	0.66	0.050	1.9	0.52
I	1.5	0.39	0.75	0.52	0.34	2.3	1.6	3.0	2.0	0.39	1.8	1.1	1.2	2.7	3.2
K	2.2	1.0	1.0	0.26	0.0093	0.42	1.1	0.48	1.6	0.0047	0.32	0.63	0.013	1.5	0.44
L	6.4	0.57	2.5	0.49	0.23	1.5	1.6	2.9	3.0	0.70	3.6	2.2	1.0	1.9	2.7
M	2.1	0.26	1.1	0.60	0.34	1.7	0.41	1.5	2.2	0.71	2.3	1.1	0.26	0.75	0.22
N	3.3	0.27	0.15	0.57	0.012	1.0	0.65	0.68	1.7	0.052	0.066	1.5	0.25	0.0049	0.62
P	0.33	0.47	0.48	0.53	0.022	0.021	0.60	1.5	0.78	0.056	0.14	1.0	0.066	2.4	0.77
Q	0.90	0.66	0.51	0.74	0.097	0.65	3.3	2.3	1.0	0.020	0.55	2.1	0.060	1.7	0.90
R	1.7	0.39	0.16	0.40	0.087	1.3	0.82	0.44	0.83	0.0038	1.3	1.5	0.064	5.2	0.21
S	0.62	0.67	0.49	0.58	0.013	1.2	1.6	0.57	3.4	0.15	0.64	2.9	0.17	2.3	0.36
T	0.78	0.93	0.41	0.71	0.010	0.98	2.5	1.0	1.4	0.049	0.41	0.45	0.40	1.2	0.21
V	0.64	0.88	0.47	1.2	0.94	2.6	1.1	0.98	1.9	0.13	2.6	1.4	1.7	5.8	0.36
W	0.63	0.69	0.52	0.61	0.42	0.83	1.3	0.40	2.6	0.58	0.50	0.67	0.24	1.3	0.45
Y	0.65	0.54	1.5	1.0	0.96	3.5	4.6	0.51	2.5	0.68	0.65	1.6	0.037	2.8	0.93
Summary²:															
Geomean	1.1	0.56	0.63	0.51	0.077	0.93	1.4	0.93	2.0	0.074	0.67	1.3	0.17	1.3	0.75
SF	0.56	1.0	0.94	1.2	7.7	0.64	0.42	0.64	0.30	8.0	0.88	0.47	3.4	0.45	0.79
10-fold effects	0	0	0	0	11	1	0	0	0	11	1	0	7	1	0