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Analysis Report

Monoclonal Antibody Development
Stage 3 Report for Project #XXXXX

The client approved using RXX rabbit to perform single B cell sorting and antibody cloning. We harvested 460 million splenocytes, sorted 60 million cells, and froze the rest into 6 cryo vials. We processed 14 8-well strips and transfected Expi293 cells with the expression DNA cassettes and obtained 40 ELISA positive clones. We then coated BSA (for background), BSA-conjugated Peptide 7 Aa, and BSA-conjugated Peptide 9 Aa to check direct ELISA binding and cross-reactivity. We also measured the antibody concentration of each sample.

As shown by the BSA ELISA, all samples exhibited minimal background binding. The BSA-conjugated Peptide 7 Aa and BSA-conjugated Peptide 9 Aa reading was color-coded with light blue to dark blue as the binding levels from weak to saturated. The 40 clones showed almost the same binding to BSA-conjugated Peptide 7 Aa as to BSA-conjugated Peptide 9 Aa. To demonstrate the correlation between color-coding and real colorimetric results, we also showed the raw picture of ELISA in the Appendix of the report. These results suggested that each of 40 clones shares similar specificity to Peptide 7 Aa and that to Peptide 9 Aa, while the latter is 2 Aas longer than the former. The measured concentrations of the samples had a mean of 1.5497 µg/mL and a median of 1.1138 µg/mL.

ELISA result readout:

Sample name in each well						Coated with BSA					
	1	2	3	4	5		1	2	3	4	5
A	[redacted]					A	0.046	0.048	0.047	0.046	0.048
B						B	0.047	0.047	0.048	0.049	0.048
C						C	0.046	0.048	0.045	0.045	0.047
D						D	0.049	0.047	0.046	0.047	0.049
E						E	0.044	0.046	0.047	0.052	0.049
F						F	0.045	0.046	0.047	0.048	0.049
G						G	0.047	0.046	0.046	0.046	0.049
H						H	0.045	0.045	0.045	0.045	0.047

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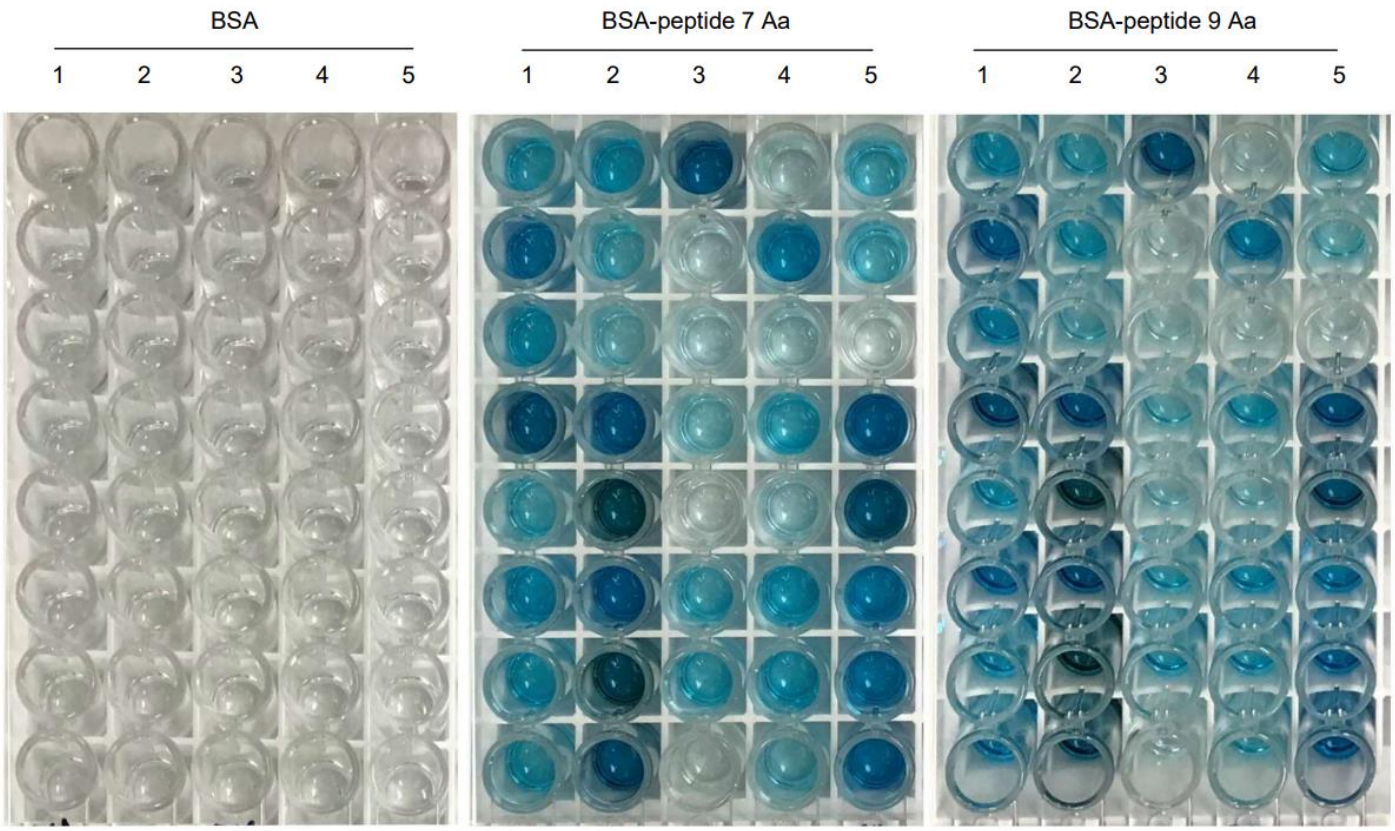
Coated with BSA conjugated Peptide 7 Aa						Coated with BSA conjugated Peptide 9 Aa					
	1	2	3	4	5		1	2	3	4	5
A	0.308	0.377	1.062	0.081	0.26	A	0.363	0.254	1.061	0.074	0.314
B	0.791	0.213	0.081	0.604	0.226	B	0.924	0.317	0.074	0.595	0.184
C	0.408	0.154	0.095	0.098	0.064	C	0.41	0.167	0.091	0.075	0.068
D	1.034	0.979	0.157	0.282	1.037	D	1.082	0.995	0.17	0.297	1.033
E	0.229	1.047	0.058	0.116	1.05	E	0.266	1.023	0.112	0.114	1.073
F	0.45	0.948	0.234	0.352	0.632	F	0.691	0.979	0.23	0.339	0.55
G	0.353	1.053	0.27	0.331	0.739	G	0.35	1.009	0.293	0.27	0.693
H	0.306	1.039	0.06	0.19	0.854	H	0.451	0.967	0.072	0.172	1.072

Antibody concentration

Antibody concentration (µg/mL)					
	1	2	3	4	5
A	0.4494	0.8454	2.1792	0.1923	1.5330
B	0.8328	2.4816	1.5681	1.9152	0.9814
C	0.3783	0.9879	1.5705	0.2938	0.4873
D	1.6800	1.8120	0.6488	0.9850	2.3709
E	1.5726	9.0989	1.1649	1.0420	3.3993
F	1.0248	1.9815	0.4334	1.0627	1.3233
G	1.3878	5.5581	0.9409	2.0577	1.7181
H	0.6039	2.0088	0.1126	0.4515	0.8520

Appendix – Pictures of ELISA results



Appendix – ELISA protocol

1. Dilute BSA conjugated peptides (7Aa or 9Aa) with 1x ELISA coating buffer (BioLegend 421701) to 1 ug/ml
2. Put 50 ul diluted antigen in each well of a 96-well plate, to cover the whole area
3. Leave at RT for > 3 hours or 4°C overnight
4. Dump the solution, and wash the wells 5 times with washing buffer (PBST, PBS with 0.05% Tween-20)
5. Block the wells by putting 120 ul PBS+1% BSA in each well
6. Incubate at RT for 1 hour
7. Dump the solution, and wash the wells 5 times with washing buffer (PBST, PBS with 0.05% Tween-20)
8. Dilute the antibody medium by 100 times with PBS+1mg/ml BSA
9. Add 50 ul diluted antibody medium to each well
10. Incubate at RT for 1 hour
11. Dump the solution, and wash the wells 5 times with washing buffer (PBST, PBS with 0.05% Tween-20)
12. Add 50ul 1:1000 diluted HRP conjugated goat anti-rabbit IgG (R&D, HAF008) to each well
13. Incubate at RT for 1 hour
14. Dump the solution, and wash the wells 5 times with washing buffer (PBST, PBS with 0.05% Tween-20)
15. Develop by adding TMB substrate