

## 7.11 Experiment Report (A)

### Experiment : ELISA Detection

**I. Experimental purpose:** After the protein was obtained, ELISA experiment was carried out to detect whether the SARS-CoV-2 antibody targeting ACE2 had a neutralizing effect on the binding of the mutant S1 protein to the ACE2 receptor.

- Plate 1: Check whether the affinity of mutant B.1.1.7 S1 and ACE2 receptor increases.
- Plate 2: Check whether the blocking effect of antibody X on the binding of mutant strain B.1.1.7 S1 and ACE2 has decreased.

### II. Experimental procedure:

#### No. 1 96-well Plates

1. Coat S1&S1-B.1.1.7 2.5ug/ml on 96-well ELISA plate, overnight at 4°C;
2. After Casein 200μl/well was sealed at room temperature for 1 hour, wash it with PBS three times;
3. Casein diluted B38&isotype (igG4), 50μl/well, incubated for 1 hour at room temperature, and washed the plate three times with 0.1% PBST;
4. Casein diluted HRP-goat-anti-human 1:2000, 50μl/well, after incubating for 1h at room temperature, wash the plate with 0.1% PBST for three times;
5. After TMB 50μl/well develop color at room temperature for about 20 minutes, stop the color development with 50μl/well 2M H<sub>2</sub>SO<sub>4</sub>, and measure the OD450 absorbance with a microplate reader.

#### No. 2 96-well Plates

1. Coat S1&S1-B.1.1.7 2.5ug/ml on 96-well ELISA plate, overnight at 4°C;
2. After Casein 200μl/well was sealed at room temperature for 1 hour, wash it with PBS three times;
3. Casein diluted B38&isotype (igG4), 50μl/well, incubated for 1 hour at room temperature, and washed the plate three times with 0.1% PBST;
4. Casein diluted HRP-goat-anti-human 1:2000, 50μl/well, after incubating for 1h at room temperature, wash the plate with 0.1% PBST for three times;
5. After TMB 50μl/well develop color at room temperature for about 20 minutes, stop the color development with 50μl/well 2M H<sub>2</sub>SO<sub>4</sub>, and measure the OD450 absorbance with a microplate reader.

		ug/ml											
coat(2µg/ml)	1st antibody	50	16.6667	5.55556	1.85185	0.61728	0.20576	0.06859	0.02286	0.00762	0.00254	0.00085	0.00028
S1-his	ACE2Fc	2.385	2.0724	2.0474	1.7411	1.3699	0.8718	0.7469	0.9442	0.6734	0.7376	0.7268	0.8524
	ACE2Fc	2.2029	2.135	1.9998	1.7928	1.2228	0.923	0.8371	0.7571	0.6852	0.7549	0.7479	0.7923
	Fc	0.4861	0.073	0.5573	0.6256	0.6604	0.6704	0.6729	0.6739	0.6904	0.6787	0.6917	0.7654
	Fc	0.6708	0.6032	0.6089	0.6905	0.6287	0.687	0.6794	0.6863	0.6849	0.6791	0.7057	0.7206
S1-his-B.1.1.7	ACE2Fc	0.4156	0.4005	0.2748	0.2088	0.1256	0.0591	0.0624	0.052	0.0708	0.0929	0.0686	0.0624
	ACE2Fc	0.4551	0.537	0.3376	0.23	0.1231	0.098	0.0876	0.0667	0.0745	0.0738	0.1373	0.0792
	Fc	0.0543	0.0403	0.0418	0.0802	0.0423	0.0489	0.0652	0.0552	0.1209	0.094	0.1095	0.0876
	Fc	0.0537	0.0608	0.058	0.0543	0.0547	0.0482	0.0607	0.19	0.1018	0.0944	0.1139	0.0947

		ug/ml												
coat	1st antibody	50	16.6667	5.55556	1.85185	0.61728	0.20576	0.06859	0.02286	0.00762	0.00254	0.00085	0.00028	
s1 WT	ACE2 (5µg/ml)	X	0.6564	0.6042	0.7101	1.041	1.8557	2.0255	2.0937	2.0919	1.9907	2.1443	2.0308	2.0937
s1 WT		X	0.6455	0.6289	0.6097	1.0098	1.7029	2.048	2.0004	2.0175	1.9291	2.0617	1.858	1.9777
s1 WT		isotype	1.0463	1.0906	1.3084	1.7995	1.8139	2.0366	1.8895	1.5678	1.8266	1.833	1.8947	1.8194
s1 WT		isotype	1.2924	0.7034	1.5551	1.7377	1.9606	1.512	1.8986	1.826	1.8484	1.809	1.5989	1.6388
s1-b117		X	0.1216	0.0875	0.079	0.1537	0.4265	1.082	2.0466	2.0427	1.9891	2.074	2.0713	2.0823
s1-b117		X	0.1003	0.0981	0.0818	0.123	0.3718	1.2748	1.6426	1.8156	2.013	1.4728	2.1092	2.1773
s1-b117		isotype	0.2399	0.391	0.4235	0.549	1.1532	1.3617	1.7434	1.5739	1.8354	1.2563	1.9033	2.1718
s1-b117		isotype	0.2594	0.2772	0.3508	0.4092	1.0797	1.1881	1.932	1.7932	1.8511	1.5678	1.662	2.1122