

7.10 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: Detect whether the affinity of B38 to mutant B.1.1.7 mutant RBD decreases, and whether the mutant B.1.1.7 has immune escape.
- Plate 2: Detect whether the affinity of mutant B.1.1.7 RBD and ACE2 receptor increases

II. Experimental procedure:

No. 1 96-well Plates

1. Coat RBD&RBD-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 H₂SO₄, and measure the OD450 absorbance with a microplate reader.

No. 2 96-well Plates

1. Coat RBD&RBD-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 ACE2Fc&hIgGFc, 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 1 hour at room temperature, wash the plate three times with 0.1% PBST;
5. After TMB 50μl/well develop color at room temperature for about 20 minutes, stop the color development with 50μl/well 2M H₂SO₄, and measure the OD450 absorbance with a microplate reader.

III. Result

	1	2	3	4	5	6	7	8	9	10	11	12
RBD+B38	1.2403	0.9288	1.2805	0.9469	0.4634	0.1775	0.1265	0.2834	0.093	0.1118	0.1119	0.0868
	1.4615	1.4309	0.9121	0.8017	0.3755	0.2171	0.1436	0.0759	0.1895	0.0823	0.0905	0.0825
RBD+ISO	0.1214	0.0675	0.1268	0.066	0.0823	0.0697	0.0874	0.072	0.111	0.082	0.088	0.0818
	0.0598	0.0731	0.0634	0.0698	0.0754	0.072	0.0723	0.0714	0.0659	0.0904	0.1046	0.1038
RBD-B.1.617+B38	1.2813	1.0022	0.8337	0.8257	0.6741	0.4146	0.1859	0.1316	0.0985	0.108	0.0935	0.0915
	1.0501	0.9607	0.6781	0.7507	0.6721	0.4019	0.2498	0.1105	0.1268	0.1044	0.0961	0.0971
RBD-B.1.617+ISO	0.084	0.0642	0.0651	0.0783	0.0733	0.078	0.0648	0.1107	0.1116	0.2916	0.0838	0.1047
	0.0789	0.0656	0.0755	0.0675	0.0685	0.0679	0.065	0.1016	0.123	0.0638	0.0876	0.196

	1	2	3	4	5	6	7	8	9	10	11	12
RBD+ACE2FC	0.9339	0.7508	0.2762	0.1275	0.1873	0.0847	0.0945	0.1427	0.1196	0.0688	0.0846	0.072
	0.0873	0.7968	0.2601	0.1283	0.0797	0.0799	0.0921	0.174	0.1564	0.0711	0.0808	0.0743
RBD+HIGGFC	1.0793	0.5383	1.2417	0.1171	0.0959	0.9374	0.3912	0.0879	0.1036	0.082	0.1087	0.2081
	0.086	0.0794	0.0775	0.0772	0.0774	0.0762	0.0712	0.073	0.0781	0.0988	0.0712	0.0808
RBD-B.1.617+ACE2FC	1.2341	0.9153	0.3861	0.1948	0.1061	0.088	0.076	0.0742	0.0769	0.0842	0.1043	0.1141
	1.3683	0.9876	0.4083	0.2191	0.1262	0.1118	0.1065	0.0771	0.078	0.1019	0.1826	0.0875
RBD-B.1.617+HIGGFC	0.0891	0.0864	0.0938	0.1002	0.0792	0.0775	0.0803	0.0794	0.0721	0.0848	0.073	0.0744
	0.0815	0.0782	0.0895	0.0961	0.2016	0.0825	0.0849	0.0835	0.0684	0.0817	0.0663	0.1434

