Protocol of SARS-CoV2 (2019nCoV) Spike-RBD antibodies standard biopanning and competitive biopanning

Standard biopanning

1. S-RBD was diluted with 0.05 M PH 9.6 carbonate buffer [1]. Then add 100 μL to each well of 96-well Maxisorp plates at 4°C overnight.

2. Aspirate the solution in the well and wash each well three times with Wash Buffer [2] (300 μL/well). Complete removal of liquid at each step is essential to good performance. Remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

3. Add 1% polyvinyl alcohol (PVA) and block at room temperature for 1 hour.

4. Repeat the aspiration/wash as in Step 2.

5. Add 100μl of phage libraries (10^{13} pfu/ml) per well for 2 hours at room temperature.

6. Repeat the aspiration/wash eight times.

7. Add 100 μL of 100mM HCl per well for 5-min incubation to elute bound phages.

8. Transfer the eluent into a 1.5ml microfuge tube and neutralized with 1M Tris-HCl (pH 8.0).

9. Mix half the neutralized phage solution with 1ml of actively growing E. coli NEB 5-alpha F' (OD600 = 0.8) in 2×YT media containing 10μg/ml tetracycline and incubated at 37°C for 1 hour.

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10. Add 10^{10} pfu of M13K07 helper phages and incubated for 1 hour.

11. The infected bacteria were amplified in 50ml 2×YT medium containing 50μg/ml carbenicillin and 25μg/ml kanamycin, shaking at 200rpm and growing overnight at 37°C.

12. Phages were harvested in precipitant with PEG/NaCl solution, then resuspended in PBS buffer for the following rounds of panning.

Competitive biopanning

1. ACE2-hFc protein was diluted to a concentration of 5μg/ml with 0.05 M PH 9.6 carbonate buffer [1]. Then add 100 μL to each well of polystyrene plate at 4°C overnight.

2. Aspirate the solution in the well and wash each well three times with Wash Buffer [2]. Complete removal of liquid at each step is essential to good performance. Remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

3. Add 1% polyvinyl alcohol (PVA) and block at room temperature for 1 hour.

4. Repeat the aspiration/wash as in Step 2.

5. Add the mixture of 10^{10} pfu antibody library and 100ng free S-RBD-His protein per well for 2-hour competitive binding at room temperature.

6. The supernatant was transferred into a 1.5ml microfuge tube containing the pre-washed Ni-NTA magnetic beads, then incubated on a shaker at room temperature for 1 hour.

7. Beads were collected using the magnetic separation rack and washed by Wash Buffer for eight times.

8. Add 100 μL of 100mM HCl per tube for 5-min incubation to elute bound phages.

9. Beads were collected using the magnetic separation rack, then transfer the eluent into a 1.5ml microfuge tube and neutralized with 1M Tris-HCl (pH 8.0).

10. Mix half the neutralized phage solution with 1ml of actively growing E. coli NEB 5-alpha F' (OD600 = 0.8) in 2×YT media containing 10μg/ml tetracycline and incubated at 37°C for 1 hour.

11. Add 1010 pfu of M13K07 helper phages and incubated for 1 hour.

12. The bacteria were amplified on the LB plates containing 50μg/ml carbenicillin at 37°C overnight.

13. Single clones were picked up for use.

[1] PH9.6 0.05M carbonate buffer:

Na2CO3 1.59 g

NaHCO3 2.93 g

Add distilled water to 1000ml

[2] Wash buffer (PH7.4 PBS):

KH2PO4 0.2 g

Na2HPO4·12H2O 2.9 g

NaCl 8.0 g

KCl 0.2 g

Tween-20 0.5ml

Add distilled water to 1000ml