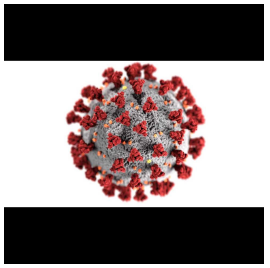
Aug 01, 2020

anti-SARS-CoV-2 spike RBD antibody discovery from phage display library



In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.bitykepw

Eve Ngoh[1], Bei Wang[1], Cheng-I Wang[1]

[1]Singapore Immunology Network, A*STAR, Singapore

Coronavirus Method Deve…

Bei Wang

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.bitykepw

Protocol Citation: Eve Ngoh, Bei Wang, Cheng-I Wang 2020. anti-SARS-CoV-2 spike RBD antibody discovery from phage display library. **protocols.io https://dx.doi.org/10.17504/protocols.io.bitykepw**

Protocol status: Working
We use this protocol in our workspace and it is working.

Created: July 21, 2020

Last Modified: August 02, 2020

Protocol Integer ID: 39512

Safety warnings

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

Bio-panning Round 1

1 Day 1: 1st round bio-panning

1.1 Add 2 µM mouse IgG_1 (as mouse Fc blocker) to theHX02 human Fab phage library (Humanyx Pte Ltd) and incubate at RT, 30min.

1.2 Wash 60 µl of DynaBeads M-280 Streptavidin (Invitrogen #11205D) with 1 ml PBS twice.

1.3 Add 100 nM of biotinylated SARS-CoV-2 RBD-mFc protein in casein to the beads and incubate at room temperature for 1 hour.

1.4 Wash beads 4 times with 1 ml PBS.

1.5 Add the pre-mix of phage library and mouse IgG_1 to the washed beads of biotinylated SARS-CoV-2 RBD-mFc.

1.6 Mix for 1 hour at room temperature.

1.7 Wash beads 7 times with 1.5 ml 0.1% PBST.

1.8 Add 250 µl of 0.1M TEA (Triethylamine) to beads at room temperature for 15 min to elute bound phage.

1.9 Collect eluted phage using magnet and neutralize pH with 125 µl of 1M Tris pH 8.

1.10 Infect neutralized phage to TG1 cells at 37°C for 45 min and spread phage infected cells onto 150 mm AG agar plates (2xYT agar, 100µg/ml Ampicillin, 2% Glucose).

1.11 Incubate agar plates at 37°C overnight.

2 Day 2: Harvest and package phages

2.1 Harvest the phage infected TG1 cells by scraping up the cells on the agar plates and let it grow to OD_{600nm} ~0.5 at 37°C, shaking at 250rpm.

2.2 Infect the bacteria culture with M13K07 helper phage (NEB #N0315S) at 37°C for 45 min.

2.3 Culture the M13K07 helper phage infected cells at 30°C for 7 hrs with shaking at 250 rpm in AKG media (2x YT media, 100 µg/ml Ampicillin, 25 µg/ml Kanamycin).

2.4 Centrifuge down the bacterial cell culture and precipitate phage (supernatant) by adding 1/5 volume of PEG (20% PEG 6000, 2.5M NaCl) to the supernatant.

2.5 Incubate at 4°C overnight.

3 Day 3: Phage preparation

3.1 Centrifuge down the PEG-precipitated phage and discard supernatant.

3.2 Resuspend the phage pellet with 500 µl of PBS so that it is ready for use for Bio-panning Round 2.

Bio-panning Round 2

4 Day 3: 2nd round bio-panning

4.1 Wash 50 µl DynaBeads M-280 Streptavidin (Invitrogen # 11205D) with 1 ml PBS twice.

4.2 During the 2^{nd} wash, split the beads into 2 tubes (tube 1 and tube 2).

4.3 Add 10nM of biotinylated SARS-CoV-2 RBD-mFc protein in casein to tube 1 beads and incubate at room temperature for 1 hr.

4.4 Wash beads 4 times with 1 ml PBS.

4.5 Add 2 µM mouse IgG_1 (as mFc blocker) to the Round 1 amplified phage and incubate at RT, 30 min.

4.6 Add the pre-mix of phage and mouse IgG_1 to the tube 2 of washed beads from Step 4.2.

4.7 Rotate at room temperature for 1 hr.

4.8 Collect supernatant (contains phage where streptavidin-binding phage have been removed by bead binding) using magnet and add supernatant to the washed biotinylated SARS-CoV-2 RBD-mFc coated beads from Step 4.4 above.

4.9 Rotate at room temperature, 1hr.

4.10 Wash beads 15 times with 1.5ml 0.1% PBST.

4.11 Add 120 µl of 0.1 M TEA (Triethylamine) to beads at room temperature for 15 min to elute bound phage.

4.12 Collect eluted phage using magnet and neutralize pH with 60 µl of 1M Tris pH 8.

4.13 Infect neutralized phage into HB2151 cells at 37°C for 45min and spread phage infected cells onto 90 mm AG agar plates (2xYT agar, 100 µg/ml Ampicillin, 2% Glucose).

4.14 Incubate agar plates at 37°C overnight.

5 Day 3: Collect the agar plates and proceed with the Fab screening by ELISA.

Binding avidity ELISA of the Fab supernatant to SARS-CoV-2 spike RBD protein

6 Day 3: Pick colonies for Fab production

6.1 Pick colonies from [Biopanning Round 2] **Step 5** into 96-well plate 1 containing 100 µl of AG media (2xYT media, 100 µg/ml Ampicillin, 2% Glucose) per well.

6.2 Shake the plate 1 at 37°C, 300 rpm for 4 hrs.

6.3 Prepare a new 96-well plate 2 with 270 µl/well of 2xYT media + 100 µg/ml Ampicillin.

6.4 Transfer 10 µl of each bacterial culture from 96-well plate 1 (**Step 6.2**) to respective wells on 96-well plate 2.

6.5 Culture the 96-well plate 2 at 37°C, 300 rpm, 1.5 hrs.

6.6 When the cultures from the 96-well plate 2 is slightly turbid, add 40 µl of 8 mM IPTG (prepared in 2xYT + 100 µg/ml Ampicillin) to each well. This will give a final 1 mM IPTG in each well.

6.7 Culture the 96-well plate 2 at 30°C overnight, 300 rpm.

7 Day 3: Coat ELISA plates.

7.1 Coat 96-well ELISA plate with 70 µl per well of 5 µg/ml NeutrAvidin Protein (ThermoFisher scientific, #31000) in carbonate coating buffer (8.4 g/L $NaHCO_3$, 3.56 g/L Na_2CO_3, pH 9.5).

7.2 Incubate ELISA plate at 4°C overnight.

8 Day 4: Continue with binding avidity ELISA using Fab supernatant

8.1 Wash each well of the ELISA plate from **Step 7** with 0.05% PBST (Tween-20), 4 times.

8.2 Block each well with 200 µl casein (Thermo Fisher Scientific, #A37528) at room temperature for 2 hrs.

8.3 Wash each well of the ELISA plate with 0.05% PBST, 4 times.

8.4 Add 70 µl of 0.2 µg/ml biotinylated recombinant SARS-CoV-2 spike protein RBD-mFc (Sino Biological, 40592-V05H) in casein per well of the ELISA casein-blocked plate. Incubate at room temperature, 1 hr.

8.5 Wash each well of the ELISA plate with 0.05% PBST, 4 times.

8.6 Add 20 µl of 7% milk (in PBS) to each well. This will give a final [milk] to 2% after addition of 50 µl Fab supernatant culture.

8.7 Centrifuge the overnight 96-well plate 2 culture from **Step 6.7** at 4000 rpm, 10min to pellet the IPTG-induced HB2151 cells. Without disturbing the cell pellet, gently transfer 50µl of each culture supernatant into respective wells in binding ELISA plate.

8.8 Incubate the ELISA plate at room temperature, 2 hrs.

8.9 Wash each well of the ELISA plate with 0.05% PBST, 4 times.

8.10 Add 70 µl of (1:3000 in casein) Peroxidase-AffiniPure F(ab')2 Fragment Goat Anti-Human IgG, F(ab')2 Fragment Specific (JACKSON ImmunoResearch, #109-036-097) in each well. Incubate the ELISA plate at room temperature, 1 hr.

8.11 Wash each well of the ELISA plate with 0.05% PBST, 5 times.

8.12 Add 70 µl of TMB One Component HRP (SurModics, #TMBW-1000-01) per well. Stop each reaction with 70 µl 1M HCl.

8.13 Measure OD_{450nm} and OD_{570nm} (baseline).

Binding avidity ELISA of the IgG antibodies to SARS-CoV-2 and SARS-CoV spike RBD proteins

9 Coat ELISA plates.

9.1 Coat ELISA plates with 70 µl per well of 5 µg/ml NeutrAvidin Protein (ThermoFisher scientific, #31000) in carbonate coating buffer (8.4 g/L $NaHCO_3$, 3.56 g/L Na_2CO_3, pH 9.5).

9.2 Incubate ELISA plates at 4°C overnight.

10 On next day, continue with binding avidity ELISA using IgG antibodies

10.1 Wash each well of the ELISA plates from **Step 9** with 0.05% PBST (Tween-20), 4 times.

10.2 Block each well with 200 µl casein (Thermo Fisher Scientific, #A37528) at room temperature for 2 hrs.

10.3 Wash each well of the ELISA plates with 0.05% PBST, 4 times.

10.4 Add 70 µl of 0.2 µg/ml biotinylated recombinant SARS-CoV-2 spike protein RBD-mFc (Sino Biological, 40592-V05H) or biotinylated recombinant SARS-CoV spike protein RBD-His (Sino Biological, 40150-V08B2) in casein into respective wells. Incubate at room temperature, 1 hr.

10.5 Wash each well of the ELISA plate with 0.05% PBST, 4 times.

10.6 Add 70 µl per well of each concentration of different clones of anti-SARS-CoV-2 spike RBD IgGs (3-fold serial dilution) into respective wells. Incubate at room temperature, 1 hr.

10.7 Wash each well of the ELISA plate with 0.05% PBST, 4 times.

10.8 Add 70 µl of (1:3000 in casein) Peroxidase-conjugated AffiniPure F(ab')2 Fragment Goat Anti-Human IgG, Fcγ Fragment Specific (JACKSON ImmunoResearch, #109-036-098) to each well. Incubate at room temperature, 1 hr.

10.9 Wash each well of the ELISA plates with 0.05% PBST, 5 times.

10.10 Add 70µl TMB One Component HRP (SurModics, #TMBW-1000-01) per well. Stop each reaction with 70 µl 1M HCl.

10.11 Measure OD_{450nm} and OD_{570nm} (baseline).

Competition ELISA of the anti-SARS-CoV2 spike RBD IgG antibodies

11 Coat ELISA plates.

11.1 Coat ELISA plates with 70 µl per well of 1 µg/ml ACE2_hFc protein in carbonate coating buffer (8.4 g/L $NaHCO_3$, 3.56 g/L Na_2CO_3, pH 9.5).

11.2 Incubate ELISA plates at 4°C overnight.

12 On next day, continue with the competition ELISA using IgG antibodies.

12.1 Wash each well of the ELISA plates from **Step 11** with 0.05% PBST (Tween-20), 4 times.

12.2 Block each well with 200 µl casein (Thermo Fisher Scientific, #A37528) at room temperature for 2 hrs.

12.3 During the 2 hours incubation time of blocking, in a separate 96-well plate, add final concentration of 0.5 nM biotinylated SARS-CoV-2 spike protein RBD-mFc with different concentrations of different anti-SARS-CoV-2 spike RBD IgG antibodies (3-fold serial dilution) in a total mixture of 100 µl per well. Incubate the plate at room temperature, 1 hr.

12.4 Wash the casein blocked ELISA plates from **Step 12.2** with 0.05% PBST, 4 times.

12.5 Add the pre-incubated mixture from **Step 12.3** at 100 µl/well into respective wells of the ELISA plates. Incubate at room temperature, 1 hr.

12.6 Wash each well of the ELISA plates with 0.05% PBST, 4 times.

12.7 Add 70 µl (1:3000 in casein) per well of streptavidin-HRP (Biolegend, #405210) to each well of the ELISA plates. Incubate at room temperature, 1 hr.

12.8 Wash each well of the ELISA plates with 0.05% PBST, 4 times.

12.9 Add 70µl TMB One Component HRP (SurModics, #TMBW-1000-01) per well. Stop each reaction with 70 µl 1M HCl.

12.10 Measure $OD_{450nm}$and OD_{570nm} (baseline).