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IgG expression and purification

In 1 collection

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1 Works for me dx.doi.org/10.17504/protocols.io.bitvken6

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PROTOCOL CITATION

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COLLECTIONS

Bivalent binding of a fully human IgG to the SARS-CoV-2 spike proteins reveals mechanisms of potent neutralization

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PARENT PROTOCOLS

Part of collection

Bivalent binding of a fully human IgG to the SARS-CoV-2 spike proteins reveals mechanisms of potent neutralization

MATERIALS TEXT

- Corning® Costar® Spin-X® centrifuge tube filters (Corning®, Cat no.: CLS8160-96EA)
- Corning® 250mL Polycarbonate Erlenmeyer Flask with Vent Cap (Qty: 50/case) (Corning®, Cat no.: 431144)
- ExpiCHO™ Expression System Kit (Thermo Scientific™, Cat no.: A29133)
- ExpiCHO™ Expression Medium (Thermo Scientific™, Cat no.: A2910001)
- ExpiFectamine™ CHO Transfection Kit (Thermo Scientific™, Cat no.: A29129)
- OptiPRO™ SFM (Thermo Scientific™, Cat no.: 12309019)
- Sartorius™ Minisart™ NML Syringe Filters, Sterile (Sartorius, Cat no.: 16534-K)
- PBS (10X), pH 7.2 (Gibco™, Cat no.: 70013032)

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- Corning® 96-well Clear Round Bottom TC-treated Microplate, Individually Wrapped, with Lid, Sterile (Corning®, Cat no.: 3799)
- Polypropylene Columns (1 ml) (QIAGEN, Cat no.: 34924)
- Protein G Agarose FAST FLOW 10mL (Merck Pte.Ltd., Cat no.: 16-266)
- Glycine powder (Merck Pte.Ltd., Cat no.: G7126-1KG)
- HyClone™ Water, Cell Culture Grade (Endotoxin-Free) (HyClone™, Cat no.: SH30529.02)
- Dulbecco's Phosphate-Buffered Saline (DPBS), 1x, w/o Ca & Mg, 500ml (HyClone™, Cat no.: SH30028.02)
- 1.0M Tris Buffer, pH 8.0, Biotechnology Grade, 1L (Axil Scientific Pte Ltd (First Base), Cat no.: BUF-1416-1L-pH8.0)
- Bio-Rad Protein Assay Dye Reagent Concentrate (Bio-Rad, Cat no.: 5000006)
- 10X Phosphate Buffered Saline (PBS), Ultra Pure Grade, 4L (Axil Scientific Pte Ltd (First Base), Cat no.: BUF-2040-10X4L)
- Slide-A-Lyzer™ G2 Dialysis Cassettes, 10K MWCO, 3 mL (Thermo Scientific™, Cat no.: 87730)
- VIVASPIN 6 30,000 MWCO PES 100/BOX (Sartorius, Cat no.: VS0622)

SAFETY WARNINGS

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

IgG Expression 1w 2d

1 Day 0: Transfection of ExpiCHO-S cells

1.1 Sterile the plasmids by filtering through 0.22 µm spin-X column.

1.2 Pre-warm ExpiCHO™ Expression Medium to 37°C in water bath.

1.3 Seed 50 ml of 6 x 10^6 cells/ml of ExpiCHO-S suspension cells in a 250 ml flask. Ensure percentage of viability is >95% before proceeding. Gently pipette cells into the medium and swirl flask to mix the cells. Put flask into incubator and prepare transfection mix.

1.4 According to the transfection manual of ExpiFectamine™ CHO Transfection Kit, 0.8 µg of plasmid per ml of ExpiCHO-S cells (20 µg of heavy chain plasmid and 20 µg of light chain) were diluted in cold OptiPRO™ SFM in a 50 ml tube.Mix by swirling the tubes.

1.5 Gently invert the ExpiFectamine™ CHO Reagent bottle 4-5 times to mix.

1.6 Add respective volume of ExpiFectamine™ CHO Reagent to the tube of diluted DNA. Mix reaction by swirling the tube.

1.7 Incubate reaction at room temperature for at least 1 minute but no more than 5 minutes.

1.8 Take out the flask of ExpiCHO-S suspension cells that was prepared in step 3 out from the incubator andadd the transfection mix dropwise into the cells while swirling the flask gently during addition.

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1.9 Place cells into back into incubator at 37°C with humidified atmosphere of 8% CO2 on an orbital shaker.

2 Day 1: Add ExpiFectamine™ CHO Enhancer and ExpiCHO Feed.

2.1 After 18-22 hours of transfection, add the required amount stated in of ExpiFectamine™ CHO Enhancer and ExpiCHO Feed.

2.2 Gently swirl the flask during addition.

2.3 Place cells into back into incubator at 37°C with humidified atmosphere of 8% CO2 on an orbital shaker for another 7 days before harvesting.

Harvest the cell supernatant containing IgGs. 1h

3 Day 8 : Harvest ExpiCHO-S cell culture supernatant

3.1 Collect transfected ExpiCHO-S suspension cells in 50 ml Falcon tubes.

3.2 Centrifuge the ExpiCHO-S suspension cells at 2000 rpm for 10 minutes.

3.3 Filter the supernatant through a 0.20 µm syringe filter to remove the cells and debris.

3.4 Add 10x PBS to neutralize pH of supernatant.

Purification of antibodies 3d

4 Day 9: Add Protein G agarose to the harvested ExpiCHO-S cell supernatant.

4.1 Wash 1ml of Protein G agarose (2 ml slurry) 3X with 5 ml of cell culture grade PBS in a 50 ml tube to remove storage buffer from Protein G agarose and add it into the supernatant.

4.2 Allow Protein G agarose to bind to the antibodies in supernatant overnight in 4°C or for 2 hours in room

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temperature with rotation before purification.

5 Day 10: Wash Protein G agarose and elute IgG antibodies

5.1 Centrifuge tube with supernatant and agarose at 150 g for 5 minutes.

5.2 Remove supernatant without disturbing the protein G agarose and collect in a new tube.

5.3 Wash 1 ml polypropylene column with cell culture grade water three times and with cell culture grade PBS for one time.

5.4 Transfer protein G agarose into column.

5.5 Wash protein G agarose with 3 column volume of cell culture grade PBS.

5.6 After washing protein G agarose with PBS, elute antibodies with 0.1 M of Glycine solution, pH 3.5 into 1.5 ml tubes with 200 µl of 1.0 M Tris, pH 8.0 to neutralise reaction.

5.7 Test 10 µl of various fractions of eluted antibody in 200 µl of 1X Bio-Rad Protein Assay Dye Reagent already prepared in a 96 well plate.

5.8 Pool the fractions of elute that turn blue in 1X Bio-Rad Protein Assay Dye Reagent together.

6 Day 10: Dialysis of antibodies

6.1 Transfer pooled fractions of elute into 10K MWCO Slide-A-Lyzer™ G2 Dialysis Cassettes and allow it to dialyse 3X, each time with 4 liters of 1x PBS prepared from 10X PBS. All buffer exchange should be done at 4°C with at least one of the dialysis done overnight.

7 Day 11: Collection of dialysed IgG antibodies

Collect dialysed antibodies from cassettes and concentrate to desired concentration using VIVASPIN 6

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7.1 30,000 MWCO if necessary.

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