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Recombinant Antibody Affinity Purification with Protein A or Protein G

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ABSTRACT

This protocol describes how to affinity purify recombinant antibodies from cell culture supernatant using Protein A or Protein G columns.

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EXTERNAL LINK

https://www.addgene.org/protocols/affinity-purification-recombinant-antibodies/

PROTOCOL CITATION

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GUIDELINES

General Considerations

Protein A and Protein G have varying affinities for species and isotypes. Refer to the manufacturer's guidelines to determine the best protein for your antibody.

Workflow Timeline

Day 1: Purify antibody

Day 2 or later: Buffer exchange

Tips and Troubleshooting:

We recommend wiping down all pipettes and equipment with 10% bleach prior to use.

MATERIALS TEXT

Equipment:

- Class II, Type A2 Biological Safety Cabinet
- 4 °C Refrigerator
- Pipette controller
- Benchtop centrifuge compatible with 50 mL conical tubes
- NanoDrop spectrophotometer
- 37 °C, 5% CO_2 incubator with shaking platform set to 120 rpm
- 37 °C bead bath
- Clamp stand and clamps
- Autoclave
- 0.1–1 mL single channel pipette
- 0.5–10 µL single channel pipett
- 20–200 µL single channel pipette
- 2–20 µL single channel pipette

Reagents and Consumables:

- Aspirating pipette, VWR 414004-265
- 5 mL pipette, VWR 89130-896
- 10 mL pipette, VWR 89130-898
- 25 mL pipette, VWR 89130-900
- 50 mL pipette, VWR 89130-902
- 50 mL conical tubes, VWR 89039-656
- Microcentrifuge tubes, VWR 89126-724
- Filtering system 0.2 µm PES, VWR 431098
- Sodium phosphate monobasic monohydrate, Millipore Sigma 71507-250G
- Sodium phosphate dibasic, Millipore Sigma S7907-500G
- 1 M Trizma hydrochloride solution pH 9.0, Millipore Sigma T2819-1L
- 250 mL sterile bottle, VWR 430281
- 0.45 µm PES complete filtration unit, 500 mL, rapid-flow, VWR 73520-984
- Syringes, 30 mL, VWR BD302832
- Filter, 0.2 µm (luer-lock), VWR 431229
- IgG Elution Buffer, Thermo Fisher 21028
- Protein G GraviTrap columns, Cytiva 28-9852-55 or rProtein A GraviTrap columns, Cytiva 28-9852-54
- LabMate PD10 Buffer Reservoir, Millipre Sigma GE18-3216-03
- 5% Sodium Azide, 500 mL, VWR 7144.8-16
- PBS, 1X pH 7.4, VWR 45000-446
- Benzamide, Millipore Sigma 12072
- Antipain, Millipore Sigma 10791
- Leupeptin, Millipore Sigma L2884
- Aprotinin, Millipore Sigma A6279
- Zeba Spin Desalting Columns, 7 kDa MWCO, 10 mL, Thermo Fisher 89893
- Amicon Ultra-15 30 kDa MWCO column, Millipore Sigma UFC903024
- 50 mL LoBind tubes, VWR 76289-498

Reagent Preparation:

1 M sodium phosphate monobasic monohydrate ($NaH_2PO_4 \bullet H_2O$), pH 7

- 138 g $NaH_2PO_4 \bullet H_2O$
- 1 L deionized water.
- Adjust pH 7
- Autoclave or filter sterilize.

1 M sodium phosphate dibasic (NaH_2PO_4), pH 7

- 142 g of $NaH_2PO_4 \bullet H_2O$
- 1 L deionized water
- Adjust pH 7
- Autoclave or filter sterilize

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1 M of sodium phosphate buffer, pH 7

- 390 mL of sterile sodium phosphate monobasic monohydrate ($NaH_2PO_4 \bullet H_2O$)
- 610 mL of sterile sodium phosphate dibasic (NaH_2PO_4)
- Store up to 1 month at 4 °C
- Adjust pH 7
- Autoclave or filter sterilize

Protein A/G binding buffer (20 mM sodium phosphate buffer, pH 7)

- 20 mL 1M sodium phosphate buffer
- 980 mL of deionized water.
- Adjust pH 7
- Autoclave or filter sterilize

1000X protease inhibitor cocktail

- 25 mg leupeptin
- 50 mg antipain
- 250 mg benzamidine
- 25 mL of 2 mg/mL aprotinin
- Mix well and sterilize through a 0.2 µm PES filter.
- Aliquot and freeze upright at -20 °C

SAFETY WARNINGS

Sodium azide is toxic. When handling, use proper personal protective equipment including laboratory coat, laboratory goggles and

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gloves. Sodium azide containing waste should be properly disposed of following your institution's hazardous waste procedures.

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BEFORE STARTING

See the Materials section for preparation of necessary stock solutions.

Warm the affinity column, binding buffer and elution buffer to room temperature before use.

Wipe down all pipettes and equipment with 10% bleach prior to use.

10m 5s

Affinity chromatography

1 Equilibrate filtered tissue culture supernatant containing the recombinant antibody to Room temperature (see Transfection for Recombinant Antibody protocol).

Note

Add protease inhibitors to 1X if this has not already been done. We typically start with about 250 mL of supernatant and add 250 µL of 100X protease inhibitor cocktail.

2 Add 1 part Protein A/G binding buffer to 1 part tissue culture supernatant and mix well.

3 Uncap the Protein G Gravitrap or rProtein A Gravitrap columns and gently pour off the ethanol storage buffer.

4 Tightly attach LabMate PD10 Buffer Reservoirs to the top of the Gravitrap column.

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Note

Gravitrap columns have a finite binding capacity. If your sample exceeds the capacity, divide the sample among multiple columns. For information about the total binding capacity for your columns, refer to the manufacturer's instructions.

5 Attach the Gravitrap column to a clamp on a clamp stand.

6 Use scissors to cut the bottom of the Gravitrap column at the indentation in the tip.

7 Equilibrate the column with 10 mL of Room temperature Protein A/G binding buffer.

8 Collect the flow-through in a 50 mL conical tube and dispose after use.

9 Carefully pour the filtered and diluted cell culture supernatant into the Gravitrap column.

10 Collect the flow through in a 250 mL bottle.

11 Repeat steps 9-10 such that the supernatant passes through the column a total of 2 times.

Note

This will ensure depletion of the recombinant antibody from the supernatant.

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12 Wash the column with 25 mL of Protein A/G binding buffer 2x.

13 Add 50 µL of 1 M Trizma hydrochloride pH 9 to 10 microcentrifuge tubes

14 Cap the Gravitrap columns at the bottom.

15 Add 5 mL of Pierce IgG Elution Buffer to the capped Gravitrap column and incubate for 00:10:00 at Room temperature . 10m

16 Uncap the column and collect 500 µL fractions into the tubes containing 50 µL 1 M Trizma hydrochloride pH 9 .

17 Cap the tubes and vortex for 00:00:05 to mix. 5s

18 Determine the protein concentration of each fraction on the NanoDrop Spectrophotometer using the A280 IgG setting.

19 Combine all eluates with measurable protein.

20 Determine the protein concentration of the pooled sample on the NanoDrop Spectrophotometer using the A280 IgG setting.

21 If the concentration of the pooled sample is above 1.0 mg/mL proceed to Step 25 with a buffer exchange using a Zeba Spin 7 kDa MWCO desalting column.

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22 If the concentration of the pooled sample is below 1.0 mg/mL proceed to Step 42 with a buffer exchange/concentration using an Amicon 30 kDa MWCO buffer exchange column.

23 (Optional) Regenerate the column by washing with 25 mL of Protein A/G binding buffer and store in 20% ethanol at 4 °C.

Buffer exchange

24 Choose Option 1 (Buffer exchange using a desalting column) or Option 2 (Buffer exchange and concentration with an Amicon column) based on the concentration of the pooled sample above.

Option 1 Buffer exchange using a desalting column

25 The 10 mL Zeba Spin Desalting Columns can accommodate up to 4 mL of recombinant antibody sample.

> Note
>
> If the volume of the sample is greater than 4 mL, then divide it into 2 columns.

26 Twist off the 10 mL Zeba Spin Desalting Column's bottom closure and loosen cap.

27 Place column in a 50 mL conical collection tube.

28 Centrifuge column at 1000 x g for 00:02:00 to remove storage solution. 2m

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29 Place a mark on the side of the column where the compacted resin is slanted upward. Place column in centrifuge with the mark facing outward in all subsequent centrifugation steps.

> Note
>
> Resin will appear compacted after centrifugation.

30 Discard the flow through into a waste container.

31 Add 5 mL PBS to the column.

32 Centrifuge at 1000 x g for 00:02:00 to remove PBS. 2m

33 Repeat Steps 31-32 3x, discarding buffer from the collection tube each time.

34 Place column in a new 50 mL LoBind collection tube.

35 Slowly apply the sample to the center of the compact resin bed.

> Note
>
> For sample volumes <1.5 mL top off the sample with 0.2 mL PBS to aid protein recovery.

36 Allow the resin bed to fully absorb the sample.

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37 Centrifuge at 1000 x g for 00:02:00 to remove PBS. 2m

38 Discard column after use.

39 Determine antibody concentration on the NanoDrop Spectrophotometer.

40 Dilute antibody to 1 mg/mL with PBS if needed.

41 For long term storage, add sterile sodium azide to 1 mM.

Option 2 Buffer exchange and concentration with an Amicon column 34m

42 Apply 15 mL of PBS to the reservoir of a Amicon Ultra-4 30 kDa MWCO column.

43 Incubate for 00:10:00 at Room temperature . 10m

44 Centrifuge at 3100 x g for 00:08:00 . 8m

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45 Discard the flow through into a waste container.

46 Add the recombinant antibody sample to the reservoir of the column.

47 Fill the remaining space in the column with PBS and pipette several times to mix.

48 Centrifuge at 3100 x g for 00:08:00 . 8m

49 Discard the flow through into a waste container.

50 Repeat steps 47-49 at least 4 additional times to ensure a full buffer exchange.

51 Discard the flow through into a waste container.

52 Fill the remaining space in the column with PBS and pipette several times to mix.

53 Centrifuge at 3100 x g for 00:08:00 . 8m

54 Remove a small aliquot of your sample from the filter reservoir to check the concentration on the NanoDrop Spectrophotometer to see if the sample has reached the desired concentration.

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55 If the sample concentration is still too low, repeat the centrifugation until the volume of the sample is reduced enough to reach the desired concentration.

Note

Periodically check the concentration on the nanodrop to see if the sample has reached the desired concentration.

56 Gently transfer the sample from the reservoir to a LoBind tube.

Note

Do not scrape the filter with the pipette tip.

57 Determine the concentration of the sample on the NanoDrop Spectrophotometer.

58 For long term storage, add sterile sodium azide to 1 mM.

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