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Coomassie Purity Stain of Recombinant Antibodies

Addgene The Nonprofit Plasmid Repository[1]

[1]Addgene

dx.doi.org/10.17504/protocols.io.8epv59255g1b/v1

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ABSTRACT

This protocol describes how to determine the purity and concentration of recombinant antibodies using ready-to-use bio-safe Coomassie G-250 stain (Addgene uses SimplyBlue SafeStain) and ImageJ software. The sample is separated by denaturing polyacrylamide gel electrophoresis alongside serial dilutions of a standard antibody of known concentration. After staining with Coomassie blue, protein band intensities are measured using ImageJ software and a standard curve is generated. The ratio of the antibody protein content to the total protein content of the sample is determined.

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EXTERNAL LINK

https://www.addgene.org/protocols/coomassie-purity-stain-recombinant-antibodies/

PROTOCOL CITATION

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KEYWORDS

recombinant antibodies, purification, Simply Blue

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60329

GUIDELINES

Workflow Timeline

- Day 1: Run SDS-PAGE and stain gel
- Day 1 or later: Image analysis

MATERIALS TEXT

Equipment:

- Heat Block
- 1-10 µL single channel pipette
- 2-20 µL single channel pipette
- 20-200 µL single channel pipette
- 200-1000 µL single channel pipette
- Pipette controller
- Microcentrifuge
- Electrophoresis chamber
- Power Supply
- Rocking platform
- Fume hood
- Metal spatula
- Razor Blade
- Plastic tray
- Gel imaging system
- ImageJ or similar software

Reagents and Consumables:

- Purified recombinant antibody, 0.9–1.1 mg/mL

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- SimplyBlue SafeStain, Thermo Fisher, LC6060
- 4-12% NuPage Novex bis-tris mini gel, 1 mm thick, 10-well, Invitrogen NP0321BOX
- 20x MOPS SDS running buffer, Invitrogen NP0001
- 4x NuPage sample buffer, Invitrogen NP0007
- 10x NuPage sample reducing agent, Invitrogen NP0009
- PageRuler Plus Prestained protein ladder, Invitrogen 26619 (optional)
- Microcentrifuge tubes, Neptune 3745.X
- Pipette tips, 1000 µL, VWR 76322-154
- Pipette tips, 10 µL, VWR 76322-132
- Pipette tips, 200 µL, VWR 76322-150
- Pipette tips, 20 µL, VWR 76322-134
- Pipettes, 10 mL, VWR 89130-898
- Pipettes, 25 mL, VWR 89130-900
- Pipettes, 5 mL, VWR 89130-896
- Pipettes, 50 mL, VWR 89130-902
- Gel loading tips, Corning 4853
- PBS, 1X pH 7.4, VWR 45000-446
- 250mL sterile bottles, Corning 430281
- Deionized water
- IgG isotype standard 2.5 mg/mL - can use commercial standard or validate an in-house standard

Reagent Preparation:

Prepare IgG Standard

- Dilute the [M]**2.5 mg/mL** IgG isotype standard in PBS as follows:
- 1.5 mg/mL: **18 µL** of 2.5 mg/mL into **12 µL** PBS
- 1.0 mg/mL: **20 µL** of 1.5 mg/mL into **10 µL** PBS
- 0.75 mg/mL: **15 µL** of 1.0 mg/mL into **5 µL** PBS
- 0.5 mg/mL: **13.3 µL** of 0.75 mg/mL into **6.7 µL** PBS
- 0.25 mg/mL: **10 µL** of 0.5 mg/mL into **10 µL** PBS
- 0.125 mg/mL: **10 µL** of 0.25 mg/mL into **10 µL** PBS
- Transfer 5 µL of each standard to a microfuge tube.
- Add 8 µL of PBS to a total volume of **13 µL** .

Prepare the recombinant antibody

- Dilute or concentrate purified recombinant antibody to a concentration of 0.9–1.1 mg/mL.
- Transfer **5 µL** of recombinant antibody to a microfuge tube.
- Add **8 µL** of PBS to total **13 µL** .

Prepare 1X MOPS buffer

- Dilute **25 mL** of 20X MOPS buffer into **475 mL** of deionized water.
- Gently invert to mix.

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BEFORE STARTING

See the Materials section for preparation of necessary reagents.

- Warm the hot plate to **100 °C**
- Thaw IgG standard and prestained protein ladder on ice.

SDS-PAGE 1h 10m

1 Add **5 µL** of 4X sample buffer to each sample.

2 Add **2 µL** 10X reducing agent to each sample.

3 Spin the sample briefly in the microcentrifuge.

4 Heat the samples for **00:10:00** at **100 °C** in a heat block. 10m

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5 Spin the sample briefly in the microcentrifuge.

6 Remove the gel from the plastic wrapper and rinse with deionized water.

7 Gently remove the white sticker on the bottom of the gel.

8 Place the gel in the electrophoresis chamber and secure it.

Check the manufacturer's instructions if you are unsure of the correct orientation of the gel.

9 Carefully remove the comb from the gel.

10 Rinse each well with **200 µL** 1X MOPS running buffer.

11 Fill the chamber with 1X MOPS running buffer.

12 Make sure that the chamber is sealed.

13 Load **5 µL** of the prestained protein ladder to the appropriate well.

When possible, skip one lane before loading samples.

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14 Load **20 µL** of each recombinant antibody sample to the appropriate well.

Leaving clear lanes between samples will make quantifying the gel easier.

15 Cover the electrophoresis chamber and attach to a power supply.

16 Run the gel at **150 V** for **01:00:00** . 1h

If the samples are running unevenly and the dye front looks like a “smile”, reduce the voltage.

17 Turn off the power supply and unplug the electrophoresis chamber.

18 Remove the gel from the chamber.

19 Use the metal spatula to gently break the gel cast open.

20 Use a razor blade to cut the top of the wells and bottom part of the gel where dye is visible.

Staining the Gel 3h 5m

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21 Place the gel in a plastic tray with **100 mL** of deionized water.

22 Rinse gel with deionized water for **00:05:00** with gentle agitation on a rocking platform. 5m

23 Pour off the water in the sink.

24 Add **20 mL** of SimplyBlue SafeStain and incubate for **01:00:00** with gentle agitation on a rocking platform. 1h

25 Pour off the SimplyBlue SafeStain in the sink.

26 Add **100 mL** of deionized water and incubate for **01:00:00** with gentle agitation on a rocking platform. 1h

27 Pour off the water in the sink.

28 Add **100 mL** of deionized water and incubate for **01:00:00** with gentle agitation on a rocking platform. 1h

29 Pour off the water in the sink.

30 Take a brightfield image of the gel with an appropriate imaging system.

Recombinant antibody preps should have 2 clear bands at ~50 kDa and ~25 kDa corresponding to the heavy chain (HC) and light chain (LC) proteins, respectively. There

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should be very little background staining.

A large shift in the electrophoretic mobility of the bands (sample AR0016 in Figure 1) indicates that the samples may not have been processed correctly in the cell (e.g., failure to cleave the signal peptide) and may not be functional. This sample would fail Addgene QC and would not be used.

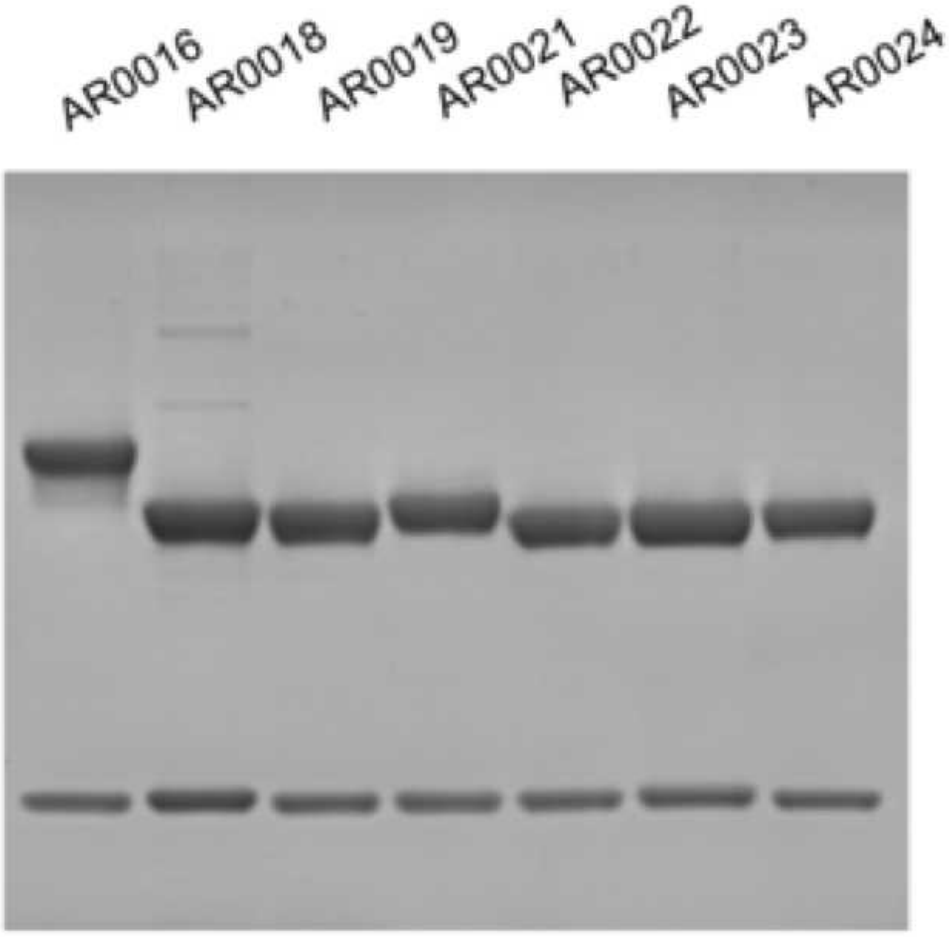

Figure 1: Brightfield grayscale image of Coomassie-stained protein gel

Image Analysis

31 Use ImageJ or a similar photo software to determine the relative intensity of the protein bands to the overall lane.

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32 Import the gel image into ImageJ.

- Select **File**.
- Select **Open**.
- Choose the location of the file to open.

33 Change the image type to **8-bit**.

- Select **Image**.
- Select **Type**.
- Choose **8-bit**.

34 Determine each lane of the gel as follows:

- Using the box tool, draw a box around the entire first gel lane (as in Figure 2).
- Select **Analyze**.
- Select **Gels**.
- Select **Select First Lane**.
- Drag the box to the next lane.
- Select **Analyze**.
- Select **Gels**.
- Select **Select Next Lane**.
- Repeat for all lanes.

Figure 2: Coomassie-stained protein gel in ImageJ photo software.

35 Plot the area under the curves for the protein bands as follows:

- Select **Analyze**.
- Select **Gels**.
- Select **Plot Lanes**.

36 One graph per lane will appear with peaks representing each protein band.

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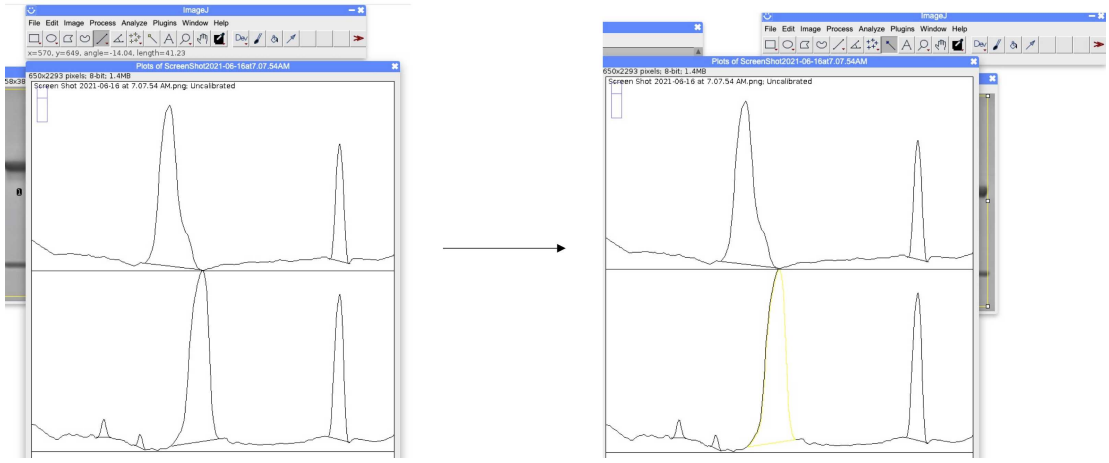

Figure 3: Histogram of protein band intensity with yellow lines outlining the area under the curve.

37 Use the line tool to connect the bottom of each peak.

38 Select the wand tool.

39 Fill in each peak by selecting the center of the peak.

40 Export results as a csv file.

- In the results table, select **File** and **Save As**.

41 Determine the percent purity of the samples.

% purity = (Area of HC + Area of LC) / (Sum of the Area of lane bands) x 100

Example for AR0018 (lane 2 in Figure 1):

A	B	C	D	E	F	G	H
Sample	Peak 1 (contaminant)	Peak 2 (contaminant)	Peak 3 (HC)	Peak 4 (LC)	Total Area	HC + LC Area	% Purity
AR0018	310.142	207.971	11469.296	4655.113	16642.522	16124.409	96.88681199

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Addgene uses recombinant antibody preps that have a purity of ≥90% but some labs use unpurified tissue culture supernatant routinely without any issues.

42 Plot the mg/mL versus the HC + LC Area for the IgG standards in Microsoft Excel.

43 Add a linear trendline for the standards.

43.1 Microsoft Excel can calculate this for you as follows:

- Select the graph.
- Select the **Chart Design** tab.
- Select the **Add Chart Element** drop down menu and select **Trendline**, **Linear**.

43.2 Determine the linear equation and the R^2 of the trendline on Microsoft Excel as follows:

- Select the graph.
- Select the **Chart Design** tab.
- Select the **Add Chart Element** drop down menu and select **More Trendline Options**.
- Select **Display Equation** on chart.
- Select **Display R-squared** on chart.

The R^2 of the trendline should be between 0.95 - 1.

44 Use the equation of the trendline to determine the concentration of your samples based on your sample's HC + LC area.

Example calculation:

If the sample HC + LC Area is 18179.442 and the linear equation of the standard curve is y =

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14725x + 3748.4, then substitute 18179.442 for y and solve for x.

18179.442 = 14725x + 3748.4
x = 0.980

The concentration of the sample is 0.980 mg/mL

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