In-gel protein staining with Coomassie Brilliant Blue

Coomassie Brilliant Blue is a pigment used to stain proteins in acrylamide or agarose gels with a detection limit of 10–20 ng per band. The staining is permanent and incompatible with Western blotting. However, Coomassie G-250 (not R-250) can also be used to visualize proteins on PVDF or nitrocellulose membranes. While silver staining SOP 0011 offers higher sensitivity, Coomassie-stained gels are compatible with downstream mass spectrometry.

Risk assessment

- Work with flammable liquids
- Work with corrosive liquids
- ▷ Wear gloves, safety glasses, lab coat
- ☐ Collect used staining and destaining solutions as HAZARDOUS WASTE

Reviewed: Jan 16, 2023

Procedures

›› *Coomassie Brilliant Blue staining*

- ☐ Incubation tray
- ☐ Lint-free wipe
- ☐ Coomassie destaining solution, 20 mL ⟨*R*⟩
- ☐ Coomassie staining solution, 20 mL ⟨*R*⟩

(1.) *Optional:* Place gel in a container with water. Microwave at 1 000 W for 1 min.

> ***This is why:*** This helps remove residual SDS and speeds up destaining. Don't boil; gels can melt or crack if microwaved dry.

(2.) Transfer gel into a tray with Coomassie staining solution. Rock gently at room temperature for 15 min. 15 min

(3.) Discard staining solution. Rinse briefly with water to remove excess dye.

(4.) Add Coomassie destaining solution. Rock at room temperature for 45 min. 45 min

(5.) Pour off the destaining solution into a collection bottle for reuse. Replace with water and add a lint-free wipe in the tray to soak up free dye. Destain overnight until background disappears.

Troubleshooting

Coomassie Brilliant Blue staining

In Step 2:

- Faint or invisible bands
  - Increase staining time or protein loading. Ensure sufficient protein was loaded. The detection limit is approximately 10–20 ng per band.
  - Verify staining solution has not been exhausted from repeated reuse.
- Uneven staining across the gel
  - Ensure the gel is fully submerged and rocking evenly. Use sufficient volume of staining solution — at least 5 times the gel volume.

In Step 4:

- High background that does not clear with extended destaining
  - Replace destaining solution more frequently. Add a crumpled lint-free wipe or activated charcoal to absorb free dye.
  - If using R-250, switch to G-250, which gives lower background due to colloidal binding.

Recipes

Coomassie destaining solution

Amount	Ingredient		Stock	Final
1.2 L	Isopropyl alcohol	[67-63-0]	100%	30%
2.4 L	Water, reagent-grade	[7732-18-5]		
0.4 L	Acetic acid, glacial	[64-19-7]	99.6%	10%

Add concentrated acid slowly to the stirred water/alcohol mixture. DO NOT autoclave. ***Note:*** The destaining solution can be reused several times and refreshed using activated charcoal. ***This is why:*** In this adaptation, 30% isopropyl alcohol or ethanol replaces the more toxic 45% methanol.

Coomassie destaining solution

30% Isopropyl alcohol, 10% Acetic acid

WARNING

Flammable liquid; Eye irritation; Skin irritation

☐ Collect as HAZARDOUS WASTE

Date: Sign: R0123

Coomassie staining solution

Amount	Ingredient		Stock	Final
1.0 g	Brilliant Blue G	[6104-58-1]	10 g/L	0.5 g/L
2.0 L	Coomassie destaining solution	R0123		

Note: The staining solution can be used multiple times. ***Hint:*** Coomassie G-250 is more sensitive while Coomassie R-250 gives sharper bands at lower cost.

Coomassie staining solution

WARNING

Flammable liquid; Eye irritation; Skin irritation

☐ Collect as HAZARDOUS WASTE

Date: Sign: R0124

Change log

2020-06-12	Hai T. Dao	Initial protocol.
2023-01-16	Benjamin C. Buchmuller	Adaptation as SOP. Replaced methanol with less toxic isopropyl alcohol.