

# Polyacrylamide Gel Staining Protocols

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## Notes

- Before pouring a gel, the acrylamide solution needs to be filtered.
- All buffers (gel-loading buffer, gel-running buffer) used for the gel running must be filtered using Millipore 0.22 micron presterilized filter.
- Totally clean the gel stain container prior to use.

## Standard Coomassie Staining

### 1. Chemical reagents

- Brilliant Blue R-250 (BBR) (Fisher BP101-25)
- HPLC water or Milli-Q water.

### 2. Solutions

- Fixing solution: 50:10:40 (methanol: acetic acid: H<sub>2</sub>O)
- Destaining solution: 45:10:45 (methanol: acetic acid: H<sub>2</sub>O)
- Coomassie concentrated stain solution: 12.0 g BBR + 300 mL Methanol dissolved, then add 60 mL acetic acid. Stir well.
- Coomassie Working solution: 500 mL Methanol+ 30 mL Coomassie concentrated stain solution + 400ml milli-Q water + 100 ml acetic acid. Mix and filter using a 0.22 micron presterilized filter.

### 3. Gel staining protocol

- a. Fix gel in fixing solution for about 30 mins.
- b. Stain gel in coomassie working solution for about 25-40 minutes.
- c. Destain until no background (shaking about 2-3 hours).
- d. Store gel in 5% acetic acid solution at 4°C until in-gel digestion is performed(Gel can be stored for several weeks).

## Gel-Code Blue Coomassie Staining

Gel-Code Blue Staining has the same sensitivity as standard Coomassie Staining. This procedure utilizes many of the features of colloidal coomassie, but the protocol is compressed into 2 main steps allowing visualization of bands within an hour. This procedure is perfect for recombinant proteins or high abundance samples.

## 1. Chemical reagents

- Gel-Code Blue stain Reagent (PIERCE Cat. 24590 or 24592)
- HPLC water or Mill-Q water.

## 2. Gel staining protocol

- a. Fix gel in fixing solution (50:10:40 / methanol: acetic acid: H<sub>2</sub>O) for 25 - 30 mins.
- b. Wash the gel with 3 aliquots of water, shaking for 5 mins each.
- c. Stain the gel in Gel-Code Blue stain Reagent for 1 hour, gently rock at room temperature.
- d. Wash the gel with ddH<sub>2</sub>O, shake about 2-3 hours, change water 3 to 4 times.
- e. Store gel in 5% acetic acid solution at 4°C until in-gel digestion is performed (Gel can be stored for several weeks).

## Colloidal Coomassie staining

This staining procedure is used for SDS-PAGE or 2D SDS-PAGE gels in which low background staining and a high degree of sensitivity is required. While having a sensitivity threshold close to that of silver staining one must keep in mind that the time required for staining is substantially greater than that of either standard silver or coomassie staining.

In order to see optimal results from this staining protocol one should allow 3 to 4 days for completion.

### 1. Chemical reagents

- Coomassie Brilliant Blue G250 (Biorad)
- HPLC water or Mill-Q water.

### 2. Solutions

- Fixing solution: 50:3:47 (ethanol: phosphoric acid: H<sub>2</sub>O)
- Neuhoffs solution: 16:25:5:54 (Ammonium Sulfate: Methanol: phosphoric acid: H<sub>2</sub>O)

### 3. Gel staining protocol

- a. Allow gels to fix in this solution overnight. (as little as 4 hours will do and fixation can be prolonged out to 4 days if needed).
- b. Removal of excess fixative and Wash gels three times for 30 minutes per wash.
- c. Equilibrate gels in Neuhoffs solution for one hour.
- d. Add Coomassie Brilliant Blue G250 powder (1 g/L) to each staining tray and stain for 3 days. Normally spots can be seen by 24-48 hours but for optimal staining stain for 3-4 days.
- e. Store gel in 5% acetic acid solution at 4°C until in-gel digestion is performed (Gel can be stored for several weeks).

## Silver Staining

(adapted from Blum et al. *Electrophoresis*, **8**, 93-99, 1987 and [UNSW Biological Mass Spectrometry and Protein Analysis Laboratory](#))

This staining protocol is compatible with mass spectrometric protein analysis (Note: not all silver staining procedures are compatible with mass spectrometry). The stain is useful for protein concentrations ranging from approximately 1 ng to 1 mg. If the staining does not work, gels can be [destained](#) and restained again (see below). It should be noted that while this method of staining is more sensitive and much faster than colloidal coomassie staining, the recovery of peptides from an in-gel digest is decreased.

### 1. Chemical reagents:

- Silver nitrate (Fisher S181-25)
- HPLC-water or Milli-Q water.
- HPLC-grade methanol
- Acetic acid
- Sodium Thiosulphate
- Sodium Carbonate
- 37% Formaldehyde
- Disodium, Ethylene Diamine Tetra-Acetic Acid (Na<sub>2</sub>-EDTA)
- Potassium Ferricyanide (for destaining)

### 2. Gel staining protocol

Prepare all solutions and reagents just prior to use!

step	reagent	final volume =100 mL	operation	time
step A	50% methanol 10% acetic acid		fix	30 min
step B	5% methanol		incubate	15 min
	milli-Q H <sub>2</sub> O		wash 3 times	3 x 5 min
step C	sodiumthiosulphate (Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> .5H <sub>2</sub> O)	0.2 g/L <b>fresh!</b> cold!	Incubate	120 sec
	milli-Q H <sub>2</sub> O		wash 3 times	3 x 30 sec
step D	silver nitrate (AgNO <sub>3</sub> )	0.2 g/100 ml <b>fresh!</b> cold!	Incubate	25 min
	milli-Q H <sub>2</sub> O		wash 3 times	3 x 60 sec
step E	sodium carbonate (Na <sub>2</sub> CO <sub>3</sub> ) 37% Formaldehyde Solution Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> .5H <sub>2</sub> O (sol.C)	3 g/100 ml 50 ul/100 ml 2 ml/100 ml	develop	10 min (max.)
step F	Na <sub>2</sub> -EDTA	(14g / L)	stop develop	10 min
	milli-Q H <sub>2</sub> O		wash	

### 3. Destaining protocol

Dissolve 0.4g potassium ferricyanide ( $K_3Fe(CN)_6$ ) in 200 ml sodium thiosulphate solution (0.2 g/L, [step c](#) above).

#### Destain:

- a. Destain until no bands are visible; the gel may be slightly yellow.
- b. Wash gel 4-5 times for 15 min with milli-Q  $H_2O$  until gel is transparent and has no background colour.
- c. Starting with [step c](#) from the gel staining protocol, restain gel.

### 4. Gel Storage

Gel can be stored in 5% acetic acid solution at 4°C for several weeks prior to in-gel digestion. Alternatively gel pieces can be stored dry (see procedure for in-gel digestion).