
Coomassie Blue staining

1. Principle

Coomassie Blue R250 is an anionic dye that stoichiometrically binds to proteins, and is therefore preferable to silver staining methods for estimation of relative abundance of proteins useful for differential expression analysis of (2-DE) gels. Although 50-fold less sensitive than silver staining, Coomassie Blue staining is a relatively simple and more quantitative method. The protocol involves soaking the gel in a dye solution. Dye that is not bound to protein diffuses out of the gel during destain steps. The proteins are detected as blue spots or bands on a clear background. This method can be used for staining proteins after both 1-DE and 2-DE. Detection limit of this method is between 50-200 ng of protein per spot.

2. Reagents and equipments

Coomassie Brilliant Blue R250 (pure)
Methanol (purity > 99,8 %)
Acetic acid (purity > 99,8 %)
Water, MilliQ (MQ)
[Glutaraldehyde (25 % solution in water)]
[Sodium acetate trihydrate (high purity)]
Ethanol (Etax A)
Whatman paper
Platform shaker
Covered tray
Gloves

3. Solutions

Coomassie R250 staining solution (0,1 % Coomassie Blue R250 (w/w), 30 % methanol, 5 % acetic acid)

To prepare 1 l of a staining solution, dissolve 1 g of Coomassie R250 to 300 ml of methanol. Then add 650 ml of MQ water and 50 ml of acetic acid. Stir the solution on a magnetic stirrer for 2 h. The solution can be filtered through a Whatman No. 1 paper to remove insoluble dye. The solution can be stored at 20 °C for several months.

Destain solution 1 (30 % methanol, 5 % acetic acid)

Add 300 ml of methanol and 50 ml of acetic acid to 650 ml of MQ water. The solution can be stored at 20 °C for several months.

Destain solution 2 (7 % acetic acid, 5 % methanol)

For 1 liter:

MQ water	880 ml
Acetic acid	70 ml
Methanol	50 ml

The solution can be stored at 20 °C for several months.

Fixing solution (0,2 % glutaraldehyde, 30 % ethanol, 0,2 M sodium acetate)

Fixing solution is needed only for small proteins and can not be used at all if proteins will be detected afterwards by a mass spectrometric method, because proteins will be fixed permanently in the gel.

For 500 ml of fixing solution:

25 % glutaraldehyde	4 ml
Ethanol	150 ml
Sodium acetate trihydrate	13.61 g
MQ water	up to 500 ml

Use immediately.

4. Instructions

Perform staining at room temperature. Covered plastic trays work well and minimize exposure to methanol and acetic acid vapors. When covers are not used, these procedures should be done in a fume hood. Wear always gloves when working with methanol. Methanol waste is collected in a waste container.

If you stain proteins for mass spectrometric identification, use always clean plastic dishes to avoid protein (e.g. BSA or keratin) contamination.

- (When staining small proteins ($M_r < 10\,000$ Da), the gel can be first fixed in a solution containing glutaraldehyde to cross-link the peptides and prevent them from diffusing out of the gel during subsequent staining steps.) NOT compatible with mass spectrometric detection!
- Submerge the gel in enough Coomassie Blue staining solution so that the gel floats freely in the tray. Shake slowly on a laboratory shaker for 30 min - 2 h. The amount of time required to stain the gel depends on the thickness of the gel. A 0.75 mm thick gel will stain faster than a 1.5 mm gel and may be completely stained in 30 min.

- Destain the gel in the destain solution 1. Change the destain solution periodically until the gel background is clear. (Alternatively, an addition of a paper laboratory wipe to one corner of the staining tray will help remove Coomassie Blue from the gel without changing the destain solution, minimizing the waste volume generated. Replace the wipes when they are saturated with dye. Use caution, however, because excessive destaining will lead to loss of band intensity.)
- Store the gel in destain 2 solution. To minimize cracking, add 1% glycerol to the last destain 2 solution before drying the gel. However, never use glycerol if proteins will be later detected with mass spectrometric methods, because glycerol will disturb ionization in a mass spectrometer.

5. Troubleshooting

Problem	Possible cause	Solution
Bands poorly stained	Insufficient staining time	Increase staining time
	Too long destaining time	Decrease destaining time
Blue background	Gel insufficiently destained	Increase destaining time or changes of destain. Use shorter staining time.