Protein Staining

Protocol

Bulletin 6209

Total Protein Staining

General protocols are described below for Mini-PROTEAN® gels. For more details, refer to the instruction manual for the stain you are using.

Bio-Safe Coomassie Stain

1. Wash gels three times for 5 min each in 200 ml diH_2O per gel.
2. Remove all water from staining container and add 50 ml of Bio-Safe Coomassie stain (or enough to completely cover gel). Agitate for 1 hr.
3. Rinse in 200 ml diH_2O for ≥30 min. Stained gels can be stored in water.

Oriole Fluorescent Gel Stain

1. If using the 5 L configuration, prepare Oriole stain solution by adding 400 ml of methanol to the 1 L bottle of diluents. Then add 10 ml of Oriole fluorescent gel stain concentrate and mix well by shaking.
2. Place gel in a staining tray with 50 ml of Oriole fluorescent gel stain. Cover the tray, place on a rocker, and agitate gently for ~1.5 hr.
3. Transfer the gel to diH_2O prior to imaging. Destaining is not necessary.

Flamingo Fluorescent Gel Stain

1. Place gel in a staining tray with 100 ml of fixing solution (40% ethanol, 10% acetic acid). Cover the tray, place on a rocker, and agitate gently for at least 2 hr.
2. Pour off the fix solution and add 50 ml of 1x stain solution (dilute 1 part Flamingo fluorescent gel stain with 9 parts diH_2O). Cover the tray, place on a rocker or shaker and agitate gently. Stain for at least 3 hr.
3. (Optional) Carefully pour off the stain solution and replace with an equal volume of 0.1% (w/v) Tween 20. Cover the tray, place on a rocker or shaker and agitate gently for 10 min.
4. Rinse gel with diH_2O prior to imaging.

TIPS

- Always wear gloves during the staining process. Try to avoid touching the gels with your fingers. Wet gloves with water or buffer before handling the gel to keep the gel from sticking and tearing
- Use clean and dust-free containers for gel staining. If possible, place a lid on the container to avoid contamination of the staining solution
- Gently agitate the container on a horizontal shaker, making sure the gel is completely covered with stain solution all the time
- Use pure chemicals and highly purified diH_2O (conductivity <2 µS)
- Fluorescent dyes like Flamingo and Oriole fluorescent gel stains have a higher dynamic range than Coomassie or silver staining techniques, making them suitable for quantitative protein analysis
- Gels stained with fluorescent dyes can be counterstained with colloidal Coomassie for further reference. In fact, doing so enhances sensitivity of the colloidal Coomassie stain
- For long term-storage, shrink-wrap the stained gels in a 10% glycerol solution (storage at 4°C) or dry them with a GelAir™ drying system within 60 min

BIO-RAD

Silver Staining (Bio-Rad Silver Stain)

Step	Reagent	Volume	Duration		
			<0.5 mm Gel	0.5–1.0 mm Gel	>1.0 mm Gel
1	Fixative 40% methanol, 10% acetic acid	400 ml	30 min	30 min	60 min
2	Fixative 10% ethanol/5% acetic acid	400 ml	15 min	15 min	130 min
3		400 ml	15 min	15 min	130 min
4	Oxidizer	200 ml	3 min	5 min	10 min
5	diH_2O	400 ml	2 min	5 min	10 min
6		400 ml	2 min	5 min	10 min
7	(Repeat washes 5–7 until all the yellow color is gone from the gel)	400 ml	2 min	5 min	10 min
8	Silver reagent	200 ml	15 min	20 min	30 min
9	diH_2O	400 ml	—	1 min	2 min
10	Developer	200 ml		~30 sec. Develop until solution turns yellow or until brown precipitate appears.	
11		200 ml	~5 min	~5 min	~5 min
12		200 ml	—	~5 min	~5 min
13	Stop 5% acetic acid	400 ml	~5 min	~5 min	~5 min

This is an excerpt from Bio-Rad's comprehensive Electrophoresis Guide (Bulletin 6040).

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