Gel Staining Procedure with Thermo Scientific PageBlue Protein Staining Solution

Protocol

This protocol is for Gel Staining Procedure with PageBlue™ Protein Staining Solution.

With microwaving (fast protocol)	Without microwaving (conventional protocol)
Total time	
25 minutes for native gels 40 minutes for SDS-containing gels	65 minutes for native gels 95 minutes for SDS-containing gels
1. Washing in water*. Repeat 3 times	
• Add 100 mL water • Microwave for 1 minute • Wash with gentle agitation for 5 minutes • Discard the water	• Add 100 mL water • Wash with gentle agitation for 10 minutes • Discard the water
2. Staining	
• Add 20 mL (or enough to completely cover the gel) of PageBlue Protein Staining Solution • Microwave for up to 30 seconds **(do not boil)** • Stain with gentle agitation for 20 minutes	• Stain with 20 mL (or enough to completely cover the gel) of PageBlue Protein Staining Solution with gentle agitation 60 minutes

*Only SDS-containing gels.

Note:

- PageBlue™ Protein Staining Solution can be reused up to 3 times without a decrease in sensitivity.
- All reagent volumes are for 8 × 10 or 10 × 10 cm minigels of 0.75-1 mm thickness. Gels should be completely immersed in solution.
- When several gels are being stained, increase the amount of staining solution accordingly.
- The first wash step is crucial to remove SDS from the gel as SDS interferes with the staining reaction.
- For staining native gels without SDS, the washing step is not required.
- Staining sensitivity can be increased if the proteins are fixed for 15 minutes either with 12% trichloracetic acid or with 25% isopropanol supplemented with 10% acetic acid. Fixation prevents protein diffusion from the gel and accelerates SDS removal. After fixation, gels can be stained immediately without additional washing.
- Using either the fast or conventional protocol, staining sensitivity is 5 ng of protein per band. To increase sensitivity to 0.05 ng per band the gel can be stained using the Thermo Scientific PageSilver Silver Staining Kit (Cat #K0681).
- For staining peptides or small proteins (more than 10 kDa) fixation of the proteins for 15 minutes either with 12% trichloracetic acid or with 25% isopropanol supplemented with 10% acetic acid is recommended. Fixation prevents protein diffusion from the gel and accelerates SDS removal. After fixation, gels can be stained immediately without additional washing. Overnight staining time is required for peptide detection.

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