Video Article

Staining of Proteins in Gels with Coomassie G-250 without Organic Solvent and Acetic Acid

Ann-Marie Lawrence[1], Hüseyin Besir[1]

[1]Protein Expression and Purification Core Facility, EMBL Heidelberg

Correspondence to: Hüseyin Besir at besir@embl.de

URL: https://www.jove.com/video/1350
DOI: doi:10.3791/1350

Keywords: Basic Protocols, Issue 30, SDS-PAGE, Coomassie staining, Protein detection, Protein staining

Date Published: 8/14/2009

Citation: Lawrence, A.M., Besir, H. Staining of Proteins in Gels with Coomassie G-250 without Organic Solvent and Acetic Acid. *J. Vis. Exp.* (30), e1350, doi:10.3791/1350 (2009).

Abstract

In classical protein staining protocols using Coomassie Brilliant Blue (CBB), solutions with high contents of toxic and flammable organic solvents (Methanol, Ethanol or 2-Propanol) and acetic acid are used for fixation, staining and destaining of proteins in a gel after SDS-PAGE. To speed up the procedure, heating the staining solution in the microwave oven for a short time is frequently used. This usually results in evaporation of toxic or hazardous Methanol, Ethanol or 2-Propanol and a strong smell of acetic acid in the lab which should be avoided due to safety considerations. In a protocol originally published in two patent applications by E.M. Wondrak (US2001046709 (A1), US6319720 (B1)), an alternative composition of the staining solution is described in which no organic solvent or acid is used. The CBB is dissolved in bidistilled water (60-80mg of CBB G-250 per liter) and 35 mM HCl is added as the only other compound in the staining solution. The CBB staning of the gel is done after SDS-PAGE and thorough washing of the gel in bidistilled water. By heating the gel during the washing and staining steps, the process can be finished faster and no toxic or hazardous compunds are evaporating. The staining of proteins occurs already within 1 minute after heating the gel in staining solution and is fully developed after 15-30 min with a slightly blue background that is destained completely by prolonged washing of the stained gel in bidistilled water, without affecting the stained protein bands.

Video Link

The video component of this article can be found at https://www.jove.com/video/1350/

Protocol

Part 1: Preparation of the CBB staining solution

1. 60-80 mg of CBB G-250 are dissolved in 1 liter of bidistilled water by stirring for 2-4 hours. Finally, 3 ml of concentrated HCl is added to the dark blue solution with stirring for another minute and stored in the dark for later use. The solution can be stored for weeks up to several months without losing its staining efficiency.
2. Concentrated HCl should be handled with the usual care und used under a fume hood. The final solution will be at about pH 2, so gloves should be used and any contact with the skin should be avoided.

Part 2: SDS-PAGE

1. Appropriate aliquots of protein samples are mixed with loading buffer to a final concentration of 1x loading buffer. We use 2x loading buffer with 125mM Tris/H3PO4 (pH 7.5 at 25°C), 2mM EDTA, 4% SDS, 200mM DTT, 0.02% bromophenol blue and 50% glycerol. Other loading buffers for SDS-PAGE can be used as well.
2. Protein samples are heated for about 5 min before loading. Meanwhile, the gel electrophoresis chamber is prepared for the run. We use precast 4-12% NuPAGE© Bis-Tris gels (Invitrogen) in a XCell SureLock® Mini-Cell (Invitrogen) with MES-buffer as the running buffer but any other gel and electrophoresis system can be used as well.
3. Protein samples are loaded on the gel and electrophoresis run for 50 min at 220V.

Part 3: Staining of the gel

1. The gel cassette is disassembled and the gel placed in a box for the subsequent washing steps.
2. About 100 ml of bidistilled water is added to the gel and heated in the microwave oven for 30 seconds. Heating should be stopped before boiling occurs. The box with the gel is then placed on a shaker for 3-5 min. and this washing step is repeated twice with fresh water.
3. Enough CBB staining solution is added to cover the gel in the box and the box heated in the microwave for 10 sec. without boiling. The box with the gel is then placed on a shaker for finishing the staining. Already after 1 minute, protein bands can be observed, after 15-30 min. the staining is strong enough in most cases.

4. The staining solution is poured off and 50-100 ml bidistilled water is added in order to further destain the light blue background of the gel on a shaker. The water can be replaced by fresh water for further destaining if needed.
5. The gel can be scanned, photographed or dried for long-term storage

Part 4: Representative Results:

See Fig. 1 for a properly stained gel following the described procedure.

See Fig. 2 for a gel that has not been washed long enough before staining and residual SDS inhibits efficient staining. Note that the marker lanes (*) contain the same amount of marker proteins.

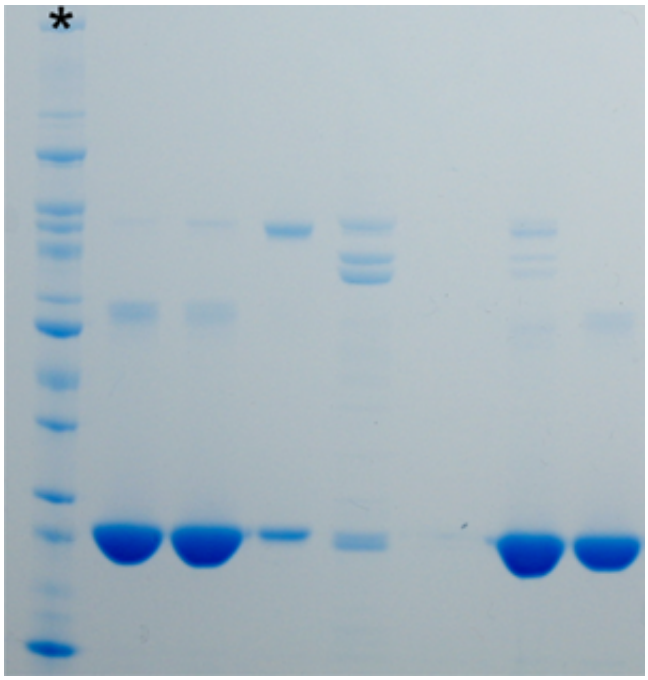

Fig. 1: Representative gel stained after loading samples of a protein purification (*: molecular weight marker).

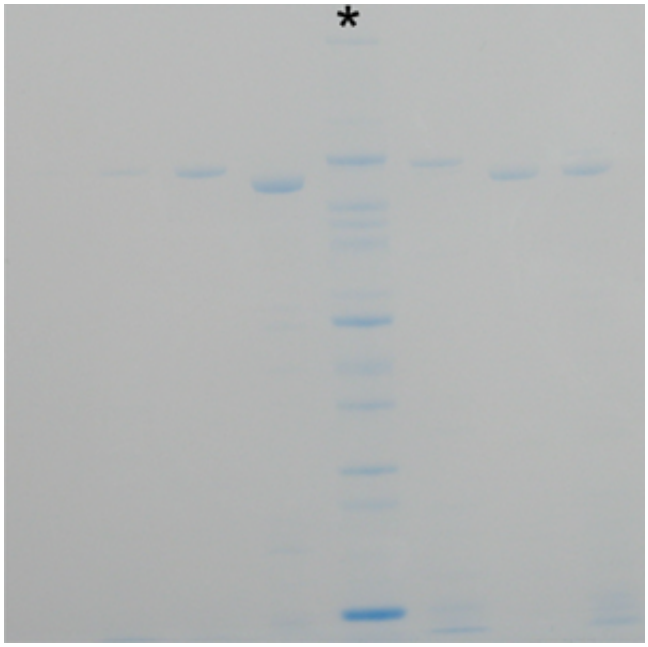

Fig. 2: CBB stained gel that has not been washed long enough before CBB staining. The protein bands appear weaker (note that the marker lane * contains the same amount of protein as in Fig. 1).

Discussion

- The washing steps are critical for the efficient staining of the proteins. A reduced washing time below 2 min or reduced volume of water (<50ml) can result in pale blue protein bands, most likely due to higher amounts of residual SDS in the gel.
- If the proteins are going to be analysed by mass spectrometry, the heating steps in the microwave oven should be skipped, the time for washing of the gel extended to about 10 min in each step and the staining time extended until the band intensity is strong enough. Heating the gel results in crosslinking of proteins to the gel matrix and thus detection of the proteins by mass spectrometry could be hampered.
- Instead of CBB G-250, one can use CBB R-250 according to the original protocol by Wondrak. We have not compared both dyes side by side as we had a stock of CBB G-250 in our lab and had good results with this dye.
- The speed of the procedure and the omission of toxic or hazardous solvents in the washing and staining steps are the most important and convincing factors for using this protocol. The sensitivity is in the same range of classical CBB staining protocols and commercial CBB staining solutions and has not been a limiting factor for our SDS-PAGE analysis in the field of expression and purification of recombinant proteins.

Disclosures

No conflicting interests. The procedure described above was originally published in a patent application by E.M. Wondrak (see Ref.).

Acknowledgements

We would like to acknowledge the technical assistance of Ines Racké.

References

1. Wondrak, E. M. (2001). Process for fast visualization of protein. (US6319720 (B1))
2. Wondrak, E. M. (2001). Solution for fast visualization of protein. (US2001046709 (A1)).

