

Tips and tricks of the trade

Coomassie Staining without Alcohol and Acetic Acid

Many researchers already leave methanol out of their recipes for Coomassie Blue staining of protein gels. However, Coomassie staining works even without ethanol and acetic acid.

Lab Hint

Dear Editor,

during my search for a fast and non-toxic procedure for Coomassie staining of protein gels, I came across a protocol published by Ewald M. Wondrak. According to his US patent specifications (US2001046709, US6555382[B2] and US6319720 [B1]), which he has obviously applied for only in the United States, one may use the following simple protocol for Coomassie staining of protein gels:

- ▶ Dilute 60 to 80 milligrams of Coomassie Brilliant Blue G-250 (CBB) in one litre of bidistilled water by stirring at room temperature for two to three hours.
- ▶ Add concentrated hydrochloric acid until an end concentration of 35 mM is reached. Approximately three millilitres of concentrated hydrochloric acid should suffice. There is no need to set the concentration exactly at 35 mM. However, it should not exceed 50 mM. Store the staining solution in the dark.
- ▶ Place the gel in a box (old plastic tip boxes work fine) and pour in the prepared Coomassie staining solution until the gel is completely covered.



Digital camera shot of a gel stained with the modified protocol. The picture has been brightened up a bit.



Leaves alcohol and acetic acid out of his Coomassie Blue staining solution: **Hüseyin Besir**

- ▶ Heat the box containing the gel in a microwave oven for about ten seconds. Do not allow the solution to boil!
- ▶ Finally, gently shake the box and check for appearing bands. Usually, the first blue-stained protein bands occur within a minute. After 10 to 15 minutes, the band intensity should be sufficient for further processing of the stained gel, e.g. documentation.

Usually, the background only turns slightly blue. To achieve a higher contrast, however, you may destain the gel background by soaking the gel in bidistilled water, without diminishing the blue protein bands. The colour of the bands actually turns a bit darker and does not vanish, even if the gel has been left in water for several days.

If you intend to further analyse your protein spots by mass spectrometry, you have to omit the heating steps during wash-

ing and staining. Perform the washing and staining steps at room temperature instead and elongate the washing procedure to five to ten minutes. If the intensity of the staining is too low, you may extend the washing time or add a further washing step.

The advantages of the presented Coomassie blue staining protocol are obvious. The staining solution is free of methanol, ethanol and acetic acid and may be heated safely in a microwave oven. Besides that, it is as fast and sensitive as traditional methods, which are often expensive and may contain hazardous compounds.

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