Lumiprobe
Life science solutions

Protein gel staining with ProteOrange

ProteOrange dye allows to quickly and easily visualize proteins after electrophoresis in polyacrylamide gel. ProteOrange is a stilbene type fluorophore containing a zwitterion fragment. Protein/sodium dodecyl sulfate micelles (SDS) selectively bind the dye in the presence of nucleic acids, polysaccharides and other molecules. Proteins with molecular weight of 6 kDa and above are efficiently stained by ProteOrange. Fluorescence signal is linear versus protein concentration over a range of concentrations covered by three orders of magnitude.

ProteOrange gel staining is approximately ten times more sensitive than Coomassie staining, although less sensitive than silver staining. For ProteOrange, detection limit is 3 ng of protein per band (detection limit for Coomassie is approx. 30 ng, for silver staining approx. 0.5 ng). However, compared to silver staining, the protocol of ProteOrange staining is much easier, because it requires no washing, and no fixing for a standard SDS-PAGE. Protocol takes 30-60 minutes to complete, and includes incubation of the gel in 7.5% acetic acid aqueous solution containing the dye, and a brief 30-second rinsing of the gel with 7.5% acetic acid. Visualization should be carried out using a transilluminator with a wavelength of 312-365 nm. Store ProteOrange stock solution at room temperature in the dark.

Protocol

1. Make sure the dye stock solution contains no precipitate. If this is not the case, keep the reagent at 70 °C for several minutes until the precipitate completely dissolves, and then shake the vial.
2. Prepare a working solution of the dye. The amount to be prepared depends on the size of gel piece and tray dimensions. For instance, 25 mL of the solution is sufficient for a 10×15 cm gel piece. To prepare this solution, dissolve 1.88 mL of glacial acetic acid in 23 mL of water to make 7.5% acetic acid, and add 5 µl of 5000x solution of ProteOrange. Stir, store in the dark for no longer than three hours.
3. After performing electrophoresis, place the gel in the tray, add the working solution of the dye, incubate for 20-60 minutes in the dark (for thinner gels or lower percentage gels, shorter times should be used; 60 min is optimal for 1 mm thick, 15% gel). Use working solutions of the dye once only, as their reuse can lead to a decreased sensitivity.
4. Rinse the gel in a 7.5% acetic acid solution (containing no dye) for 30 seconds. The gel is ready for visualization.
5. For visualization, place the gel onto a transilluminator.
6. To remove the dye, incubate the gel for 10-12 hours in a 0.1% solution of Tween 20 or repeatedly wash it with 7.5% acetic acid solution.

Notes

- To improve sensitivity, we recommend to perform electrophoresis with 0.05% SDS (vs standard 0.1%) added to the TGB buffer. This change in SDS concentration does not affect mobility of proteins, but reduces time of staining.
- Do not fix gel before staining in methanol/ethanol containing solutions.
- For small proteins or low percentage gels, 10% solution of acetic acid should be preferred.
- The dye is not suitable for staining proteins after transferring them to a membrane.
- In case gel is to be used in Western blotting, ProteOrange staining can be carried out in a standard transport buffer, but this will lead to a decrease in sensitivity.
- For Triton X-100 gel electrophoresis, wash gel in Triton X100 free TGB buffer (3×20 min) as soon as electrophoresis is finished, and shortly thereafter incubate the gel in TGB buffer with 0.05% SDS added for 30 minutes prior to staining.
- To stain protein during electrophoresis, ProteOrange should be dissolved in the upper (cathode) buffer. After electrophoresis is finished, gel should be incubated in a 7.5% acetic acid solution for 30 min to reduce the background level of fluorescence.
- Staining gels without SDS is possible, but the method becomes less sensitive, and the sensitivity largely depends on amino acid composition of the proteins. Unless native proteins should be recovered after electrophoresis, the gel should be incubated in a buffer with 0.05% SDS, and stained according to the standard protocol.
- Normally, prestained protein ladders do not fluoresce when they are further stained with ProteOrange. Use unstained ladders only.
- After staining with ProteOrange, Coomassie or silver staining is still possible.

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