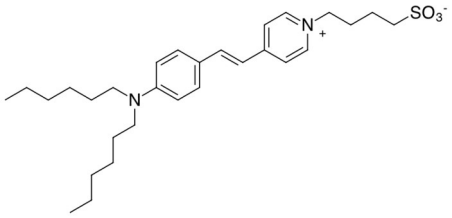
Protein Gel Stain - ORANGE, 5000X

Protein-binding dye for staining of protein gels

Products Description

Name :	**Protein Gel Stain - ORANGE, 5000X in DMSO**
Catalog Number :	**FP-76541A**, 1ml
Absorption / Emission:	$\lambda_{exc}\backslash\lambda_{em}$ = 470/ 570 nm
Storage:	Room temperature



Introduction

Protein Gel Stain - ORANGE is a protein-binding dye for staining of protein gels. The fluorophore is a styrene-like zwitterionic dye. It binds proteins selectively in the presence of nucleic acids, polysaccharides, and other molecules. It is also tolerant to SDS, and therefore can be used for SDS-PAGE gel staining. The dye interacts with the SDS coat around proteins in the gel, so it gives more consistent staining between different types of proteins compared to CBB staining, and never exhibits negative staining. It also stains glycoproteins well.

Staining with Protein Gel Stain - ORANGE is about an order of magnitude more sensitive than Coumassie dye staining, and allows to detect about 3 ng of protein per band (30 ng is detection limit for Coumassie).

Protein Gel Stain - ORANGE is supplied as a 5000x solution in DMSO. 1 mL of the solution is enough for the staining of around 200 minigels.

Technical and scientific information

Directions for use

Protein Gel Stain – ORANGE dye allows to quickly and easily visualize proteins after electrophoresis in polyacrylamide gel. Protein Gel Stain - ORANGE 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 Protein Gel Stain - ORANGE. Fluorescence signal is linear versus protein concentration over a range of concentrations covered by three orders of magnitude.
Protein Gel Stain - ORANGE staining is approximately ten times more sensitive than Coomassie staining, although less sensitive than silver staining. For Protein Gel Stain - ORANGE, 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 Protein Gel Stain - ORANGE 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 Protein Gel Stain - ORANGE stock solution at room temperature in the dark.

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 10x15 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 Protein Gel Stain - ORANGE. 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.

Note

- 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, Protein Gel Stain - ORANGE 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 (3x20 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, Protein Gel Stain - ORANGE 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 Protein Gel Stain - ORANGE. Use unstained ladders only.
- After staining with Protein Gel Stain - ORANGE, Coomassie or silver staining is still possible.

References

Shukla D. *et al.*, Characterization of Self-Assembled Protein Scaffolds from Collagen-Mimetic Peptides, *MIMB*, volume 1798, Protein Scaffolds pp 223-237 (2018)

Related / associated products and documents

• Acrylamide/Bis-Acrylamide 29:1, 40 % UP864927 • SDS 20 %, 896828

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com.
Please inquire for higher quantities (availability, shipment conditions).

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