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Product Information

EZBlue™ Gel Staining Reagent

Catalog Number **G1041**
Storage Temperature 2–8 °C

Product Description

EZBlue™ Gel Staining Reagent is a ready-to-use, Coomassie® Brilliant Blue G-250 based protein stain for ultrasensitive detection on polyacrylamide gels, and nitrocellulose or PVDF membranes. EZBlue stains only proteins while leaving the gel background relatively clean so the protein bands can be viewed directly during the staining process. This visualization of the bands saves time since the staining can be stopped when the proteins have been adequately stained. A destaining step is not required as with other Coomassie-based stains, but rinsing the gel with water following the staining step will enhance the sensitivity.

EZBlue Gel Staining Reagent is a ready-to-use solution containing Brilliant Blue G-250 at a low pH and can detect 5 ng of protein.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

EZBlue Gel Staining Reagent is a ready-to-use solution.

Storage/Stability

The product remains active for at least one year stored at 2–8 °C in an unopened container.

Procedures

A. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE Gels)

1. Gel rinse - After electrophoresis, place the gel into a clean container. Rinse the gel 3 times for 5 minutes each with an excess of water. This removes the SDS in the gel that causes background staining.

2. Gel fixation - Fixing steps are usually not required in standard Laemmli[1] gels, but the use of buffers other than Tris-HCl requires a fixing step using 50% methanol/10% acetic acid or Fixing Solution (Catalog No. F7264). The proteins should be fixed with either solution for 15 minutes.

3. Gel rinse - If a fixing step was used, wash the gel for 10–15 minutes with an excess of water to remove the fixing solution before staining the gel.

4. Gel staining - Add the EZBlue Gel Staining Reagent to the container. The amount of staining reagent required for the gel will depend on the size of the container used. Typically, 20–40 ml of reagent will be sufficient for a mini-gel. Gently shake the container and periodically check the protein band development. Staining intensity generally reaches a maximum within 45 minutes to 1 hour. Staining overnight will not increase the background.
   Note: PhastGel® gels may require longer staining times (2 hours to overnight) or a higher temperature (37–50 °C) for optimal band visualization.

5. Gel wash with water - If there is remaining SDS in the gel, the background may be slightly blue. The gel should be washed with water until the background becomes clear. Change the water often to enhance the contrast between the stained protein and the background. Lighter protein bands tend to increase in color during this step. Full background destaining should take only about 1–2 hours at room temperature. Staining can be achieved faster if the temperature is increased. The gel can be left to destain overnight without any loss of sensitivity. Thicker gels (>1 mm) will have a slight blue background due to remaining SDS in the gel and may require a slightly longer water wash time.

B. Native PAGE Gel

1. Gel rinse - Rinse the gel once with water for 5 minutes.

2. Gel Fixation - Native gels run in a Tris-glycine buffer do not normally require a fixing step. However, if poor staining or high background is observed, run the gel again and fix with 50% methanol/10% acetic acid or Fixing Solution (Catalog No. F7264).

3. Gel rinse - If a fixing step was used, wash the gel for 10–15 minutes with a large excess of water to remove the fixing solution before staining the gel.

4. Perform gel staining and water wash as indicated in steps 4 and 5 of the SDS-PAGE Gels procedure.

C. Western Transferred Proteins - PVDF or Nitrocellulose Membrane Staining

1. Protein Transfer - Transfer the proteins from the gel to a PVDF or nitrocellulose membrane using standard procedures.

2. Membrane Rinse - Rinse the membrane containing transferred proteins for 1–2 minutes with water.

3. Perform membrane staining and water wash as indicated in steps 4 and 5 of the SDS-PAGE Gels procedure.

4. Membrane wash enhancement step - Washing with a 20% methanol solution will help to decrease the background staining on the membrane. Multiple changes of this methanol solution may be necessary to obtain the desired background. The solution may be changed every 5–10 minutes. Use only enough solution to keep the membrane floating free in the container. Excessive washing may remove dye from the protein bands.

D. Isoelectric Focusing Gels (IEF)

1. Gel fixation - After electrophoresis, place the gel into a clean container. Add Fixing Solution (Catalog No. F7264) to completely cover the gel. Allow the gel to stand in the Fixing Solution for 15 minutes to fix the proteins and remove the ampholytes.

2. Gel rinse - The gel should be washed for 10–15 minutes with an excess of water to remove the Fixing Solution before staining the gel.

3. Perform gel staining and water wash as indicated in steps 4 and 5 of the SDS-PAGE Gels procedure.

References

1. Laemmli, U.K., Nature, **277,** 260 (1970).

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