Brilliant Blue R250

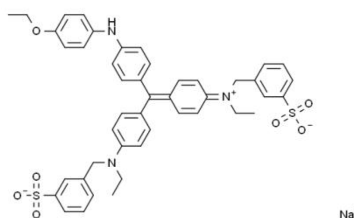
Cat: IB6730
Storage: RT, 2 years

Introduction

Brilliant Blue R250 is an anionic dye that is most commonly used to detect proteins dissolved in SDS-PAGE gels. The staining sensitivity of Brilliant Blue R250 is 5 times higher than that of amino black. It is especially suitable for SDS electrophoresis micro-protein staining, which shows basically the same color when combined with different proteins, and in a relatively wide range, the area of scanning peak is linear with the amount of protein. However, the staining of high concentration protein does not conform to Beer's law when the protein concentration exceeds a certain range, which should be noted when used for quantitative analysis.

Parameter

CAS: 6104-59-2
Molecular Formula: $C_{45}H_{44}N_3NaO_7S_2$
Molecular Weight: 825.97
Appearance: Solid



Protocols *(only for reference)*

1. Working Principle

Through electrostatic binding interactions with amino and carboxyl groups within proteins as well as VDW(Van der Waals' force), Brilliant Blue R250 forms strong complexes with proteins, but is not covalently linked. The formation of protein-dye complexes stabilizes the negatively charged anions (such as sulfonate groups) carried by the dye to produce a blue color that is visible to the naked eye on the membrane or on the gel.

2. Preparation of staining solution

A: Add 100 mL of glacial acetic acid to 450 mL of ultrapure water.
B: Dissolve 3 g of Brilliant Blue R250 in 450 mL of methanol.
The AB solution was mixed thoroughly and filtered to obtain 1xR-250 working solution.

Preparation of decolorizing solution

Mix 250 mL methanol, 80mL glacial acetic acid, and volume to 1000 mL with ultrapure water.

Note: Staining solution and decolorizing solution can be reused. Configured staining and decolorizing solutions can be stored at room temperature or 2-8°C for several months.

3. Standard dyeing procedure

1) Protein electrophoresis gels were fixed in 50% methanol, 10% acetic acid, and 40% aqueous solution, and shaken at low speed on a horizontal shaker for more than 30 min.

2) Remove the fixative and replace the gel with 0.25% Brilliant Blue R250 staining solution (0.25% R250 dissolved in 50% methanol, 10% acetic acid, and 40% aqueous solution) to cover the

gel completely, and stain the gel for 2-4 h until the gel stains a uniform blue color.

Note: Staining was complete when the gel was not visible to the naked eye within the staining solution, otherwise the gel area was looked light in color when compared to the darker stain.

3) Place the gel in 5% methanol, 7.5% acetic acid, 87.5% aqueous solution to decolorize the gel for 4-24 h. Protein bands can be seen after about 1-2 h, and decolorization was not finished until the background becomes transparent. The decolorizing solution can be changed 2-4 times in between.

4) Store the gel in 7% acetic acid. It can also be stored in water or dry.

4. Rapid dyeing procedure

1) Protein electrophoresis gels were fixed for 30-60 min in an aqueous solution of 25% IPA, 10% acetic acid.

2) Stain the gel by placing it in an aqueous solution of 10% acetic acid (containing 60 μg/mL of Brilliant Blue R250). After about 30 min, the bands were shown and the gel was allowed to continue staining until the desired protein bands were achieved. Due to the low concentration of gel used, the background staining of the gel was lighter in this staining method.

3) Put the gel back in 10% acetic acid solution to decolorize for 2 h or longer, during which the decolorizing solution can be replaced 2-4 times.

4) Store the gel in 7% acetic acid. It can also be stored in water or dry.

Note

1. For your safety and health, please wear lab coat and disposable gloves.

2. This product is for scientific research use only. Do not use in medicine, clinical diagnosis or treatment, food and cosmetics. Do not store in ordinary residential areas.