



Safe Red Stain

Product Number: ST05

Shipping and Storage

Can be stored for 24 hours in dark at 4°C or room temperature.

Description

Safe Red Stain is the third generation non mutagenic safe nucleic acid dye, with electrophoretic performance surpassing that of the first generation toxic nucleic acid dye and the first generation toxic nucleic acid dye. Strict testing by the WuXi AppTec has proven its safety, toxicity, mutagenicity, clear and complete band shape, good migration rate, accurate quantification, uniform staining, high sensitivity, stability, and dosage>1000.

Features

1. Clear and neat band shape: The third generation nucleic acid dye Safe Red Stain completely overcomes the disadvantage of original foreign similar products in terms of dispersion of single-segment DNA bands, and the electrophoretic bands are clear and neat, making them aesthetically pleasing.
2. Safe toxicity: The unique oily component prevents it from penetrating the cell membrane and entering cells. Ames test experiments have shown that this dye does not have the carcinogenic mutagenicity of EB.
3. Good migration rate: EB particles quickly escape from the gel, so EB can easily cause unclear DNA segments. Our Safe Red Stain completely overcomes this issue.
4. Quantitative accuracy: Suitable for the determination and quantification of nucleic acid fractions, EB is not accurate in quantifying DNA segments.
5. Evenly dyed: Evenly dyed during electrophoresis, near the negative gel and near the positive extreme brightness samples. EB can cause the overall background of the glue to be slightly darker, often with a yin and yang background (some of the glue background is bright and some is dark); Electrophoresis with EB time and distance will result in a corresponding decrease in EB signal intensity. Our Safe Red Stay completely overcomes this point.
6. Sensitivity: Suitable for electrophoretic staining of various DNA segments, with the same nucleic acid mobility as EB, comparable to SYBR Green I.
7. Stability: heat-resistant, can be added to buffer solution, dissolve gel at 100 °C, and anti staining agent is not fully mixed. Suitable for preparing agarose gel by microwave or other heating methods; Extremely stable in acidic or alkaline buffer at room temperature.
8. Strong light resistance: Under normal light conditions in the laboratory, it can be stored in a brown frozen storage tube for 24 hours at room temperature.
9. Good signal-to-noise ratio: The sample has a strong fluorescence signal and a low background signal. The fluorescence brightness is more than twice that of EB, and the brightness can be clearly observed by the naked eye. EB can cause the overall background of the adhesive to slightly darken, often with a negative and positive background.
10. Simple operation: completely identical to the EB method, the dye does not degrade during pre gelation and electrophoresis processes; After electrophoresis, the staining process also takes only 30 minutes and the staining process needs to be removed or washed, which can be directly observed by the ultraviolet gel transmission instrument.
11. Suitable range: You can choose pre electrophoresis staining (gel staining method) or post electrophoresis staining (bubble staining method); Suitable for agarose gel or polyacrylamide gel electrophoresis; Can be stained with dsDNA, ssDNA, or RNA.
12. Perfect compatibility: It has the same spectral characteristics as EB, and needs to change the filter light and observation device: the standard EB filter light or SYBR filter light are both suitable for observation, so that it can be observed by the ordinary ultraviolet gel transmission instrument which is the same as EB, and the best excitation can be obtained near the 300nm

ultraviolet light.

Protocol

1. Gel dyeing method (recommended method, similar to EB)

When making glue, add Safe Red Stain nucleic acid dye (dye sensitive, add 10 μ L of Safe Red Stain original solution to every 100mL of agarose solution). Follow the conventional electrophoresis method.

1.1. Laboratory materials and reagents:

1.1.1. Experimental sample: Plasmid DNA, DNA marker (although the concentration of domestic DNA markers is relatively high, they can be used in normal amounts of 5 μ L.)

1.1.2. TAE buffer configuration: 50 \times TAE electrophoresis buffer [Tris 242g (2M), EDTA 37.2g (100mM), add about 57ml of acetic acid, adjust pH=8.5; constant volume 1000mL]; Dilute ddH₂O 50 times to prepare 1 \times TAE electrophoresis buffer.

1.1.3. TBE buffer configuration: 10 \times TBE electrophoresis buffer [Tris 107.8146g (890mM), boric acid 55.0287g (890mM), EDTA 5.845g (20mM), add about 4g of NaOH to adjust pH=8.3; constant volume 1000mL]; Dilute ddH₂O 10 times to prepare 1 \times TBE electrophoresis buffer.

1.1.4. Bromophenol blue indicator, 1% Spanish agarose gel.

1.1.5. Instrument: electrophoresis apparatus (130v), pipette (0.5~10 μ l), gel imager

1.2. Protocol

1.2.1. Gel making: Dissolve 0.5g of agarose in 50mL of 1TAE electrophoresis buffer, and heat until the agarose completely melts. Place the fused agarose solution at room temperature at about 50°C, add 5 μ l of Safe Red Star gel electrophoresis dye, and shake well.

1.2.2. Pour glue: slowly pour the prepared agarose gel into the glue making tray to avoid producing bubbles. Place the sample comb vertically at the end of the electrophoresis gel film, approximately 1mm from the bottom of the tray. Try to maintain stability and avoid shaking when placing.

1.2.3. Glue placement: After the gel has fully solidified for about 30 minutes, slowly and vertically pull out the starting sample comb, and do not use excessive force. (The gel time should be extended appropriately in summer).

1.2.4. Put the agarose gel into the electrophoresis tank, add the electrophoresis buffer solution, and make the electrophoresis buffer solution immerse in the gel for about 1-2 mm.

1.2.5. Add a DNA sample of mixed bromophenol blue indicator (1 μ l bromophenol blue mixed with 5 μ l DNA sample) to the sampling well.

1.2.6. Cover the electrophoresis tank and turn on the power to move the DNA from the negative electrode to the positive electrode for constant voltage electrophoresis (voltage constant between 120-130V, generally 130V can be selected).

1.2.7. Turn off the power when the DNA strip is about 1~2cm away from the spot hole, and take out the gel (about 30~40 minutes).

1.2.8. The results were observed with a 302 nm excited UV gel imaging system.

Note: The amount of dye used in this method is relatively small. Dyes added to the glue can be directly heated in a microwave oven, and the prepared glue solution can be stored at room temperature for a long time.

1.3. Reference items for electrophoresis conditions:

1.3.1. Because EB is inserted into DNA as a whole, it is not prone to migration/dispersion issues. Although our Safe Red Stain and DNA are covalently bound through electrostatic attraction, we have completely overcome the problem of large amounts of DNA diffusion caused by similar foreign products!

1.3.2. Although most domestically produced DNA markers have a relatively high concentration, our Safe Red Stain is still suitable after testing, and there is no need to dilute the marker twice as much as similar foreign products!

1.3.3. Replace the electrophoresis buffer, the newly configured electrophoresis solution works well! TBE buffer solution has better performance than TAE because reagents containing borate have better conductivity.

1.3.4. The voltage should not be too high during electrophoresis, generally not exceeding 130V.

- 1.3.5. The migration rate and band separation effect of DNA are similar to those of EB in Safe Red Stain, completely similar! However, EB can cause the overall background of the adhesive to slightly darken, often appearing as a negative or positive background (the background of the adhesive is partly bright and partly dark). This method can distinguish whether the dye is configured with EB.
- 1.3.6. After the Safe Red Star dye is mixed with the sample, the sample is injected into the agarose gel. This method is not recommended.
- 1.3.7. Due to its good thermal stability, Safe Red Stain can be directly added to a hot agarose solution without waiting for the solution to cool. Shake, shake, or flip to ensure thorough mixing of the dye. You can also choose to add the Safe Red Star storage solution to the agarose powder and electrophoresis buffer, and then heat it in the microwave oven or other normal way to prepare agarose gel. Safe Red Stay is compatible with all commonly used electrophoretic buffer solutions.
- 1.3.8. If you always see band dispersion or unsatisfactory separation, it is recommended to dye the gel after electrophoresis to avoid potential interference of dyes on DNA migration. Use bubble dyeing to confirm if the problem is related to the dye. If the problem still exists after dyeing, it indicates that the problem is related to the dye!

Note: This method is not suitable for prefabricating polyacrylamide gel. For polyacrylamide gel, please use the soaking staining method.

2. Bubble staining method

- 2.1. Perform electrophoresis according to the conventional method. The bubble staining method is strongly recommended for high concentration DNA samples such as gel recovery!
- 2.2. Dilute the Safe Red Stain storage solution by approximately 3300 times with H₂O into a 0.1M NaCl solution to prepare a 3×dye solution. (For example, add 15μL of Safe Red Stain storage solution to 50mL of 0.1M NaCl solution).
- 2.3. Put the gel into a suitable container, such as polypropylene container. Slowly add a quantity of 3×dye solution to immerse gel. The optimal staining time was slightly different according to the thickness of gel and the concentration of agarose. For gel containing 3.5-10% acrylamide, the dyeing time is usually between 30min and 1h, and the dyeing time is prolonged with the increase of acrylamide content.
- 2.4. The results were observed with a 302 nm excited UV gel imaging system.

Note: When using bubble dyeing method, there is a large amount of dye used. A single use of the dye solution can be repeated about 3 times; The 3-Safe Red Stain dye solution can be prepared in large quantities and stored in dark at room temperature until complete.

3. PAGE steps for nucleic acid electrophoresis:

- 3.1. Put the gel prepared by TBE into the electrophoresis tank, and clamp the edge.
- 3.2. Fill the buffer tank with 5×TBE of the same batch of gel solution. Use a syringe to remove the bubbles at the bottom of gel.
- 3.3. Use a syringe to suck 1×TBE and rinse the sampling hole. Mix the DNA sample with an appropriate amount of 6×gel loading buffer, and add it to the sampling hole with a micropipette.
- 3.4. Connect the electrode to the power supply (connect the positive electrode to the lower slot), and turn on the power supply, usually 90V; 1-8V/cm. Perform electrophoresis for 9 hours.
- 3.5. The electrophoretic standard refers to the required position for dye migration (usually when electrophoretic until toluene is completely removed, and bromophenol blue stops 2-3 cm from the bottom). Turn off the power, unplug the plug, and discard the electrophoresis solution from the electrophoresis tank.
- 3.6. Take down the gel and put it into the dye solution. Add 30-60 minutes of shaking dye in 3-Safe Red Star's 1×buffer solution and place it in the UV detection.

Note: unlike agarose gel, pre staining or spot staining can not be used; Only the foam dyeing method can be used to show the gel. Because the polyacrylamide gel is dense, the dye is not easy to penetrate, and the effect of showing gel is not as good as that of agarose gel.