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Safe Green Stain

Product Number: ST04

Shipping and Storage

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

Features

1. Safe and non-toxic: The unique oily macromolecules prevent it from penetrating the cell membrane and entering the cell. This dye does not have EB carcinogenic mutagenicity.
2. Good migration rate: EB small molecules quickly escape from the gel, so EB can easily cause small DNA fragments to be unclear. Our large molecule Safe Green Stain completely overcomes this.
3. Accurate quantification: Suitable for determining and quantifying the size of nucleic acid molecules, EB is not accurate in quantifying small DNA fragments.
4. Uniform dyeing: the brightness of the negative and positive ends of the whole gel is the same. EB can cause the overall background of the glue to be slightly higher, often appearing as a yin and yang background (the background of the glue is partly bright and partly dark); Long term and long-distance electrophoresis of EB will result in a corresponding decrease in EB signal intensity. Our macromolecular Safe Green Stain completely overcomes this.
5. High sensitivity: suitable for electrophoretic staining of various sizes of fragments, with less impact on nucleic acid migration than SYBR Green I.
6. High stability: heat resistant, can be added in buffer solution, and gel can be dissolved at 100 ℃ to prevent dye from not being fully mixed. It is suitable for preparing agarose gel by microwave or other heating methods.
7. Strong light resistance: It can be stored at room temperature for 24 months under daily light exposure in the laboratory.
8. Good signal-to-noise ratio: The sample has a strong fluorescence signal, a low background signal, and a fluorescence brightness more than ten times that of EB. The brightness can be observed with the naked eye to be significantly stronger than EB.
9. Easy to operate: The usage is exactly the same as EB, and the dye does not degrade during the pre gel and electrophoresis process; The staining process after electrophoresis only takes 30 minutes and does not require decolorization or rinsing.
10. Wide scope of application: suitable for agarose gel or polyacrylamide gel electrophoresis; Can be used for staining dsDNA, ssDNA, or RNA.
11. Perfect compatibility: Safe Green Stain is compatible with all ultraviolet g el transmission instruments; Compatible with all blue and visible light instruments. It has similar spectral characteristics to EB, and does not need to change the filter and observation device: standard EB filter or SYBR filter are applicable, and the best excitation can be obtained near 300 nm ultraviolet light by using the same ultraviolet gel transmission instrument as EB.

Protocol

Gel dyeing method (pre dyeing method, with the same usage EB)

When making glue, add Safe Green Stain (dye sensitive, add 10μl of Safe Green Stain original solution to every 100mL of agarose solution). Perform electrophoresis according to conventional methods.

1. Laboratory materials and reagents:
   1.1. Experimental samples: Plasmid DNA, DNA marker (domestic DNA markers have a high concentration, diluted at least 2-3 times before use)
   1.2. TBE buffer configuration: 10X 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 10 times with ddH2O to prepare 1X TBE electrophoresis buffer.
   1.3. TAE buffer configuration: 50X TAE electrophoresis buffer [Tris 242g (2M), EDTA 37.2g (100mM), add about 57ml of

acetic acid to adjust pH=8.5; constant volume 1000mL]; Dilute 50 times with ddH2O to prepare 1X TAE electrophoresis buffer.

1.4. Bromophenol blue indicator, 1% Spanish agarose gel, electrophoresis instrument (130v), pipette (0.5~10μl), gel imager

2. Protocol:

2.1. Gel making: Dissolve 0.5g of agarose in 50mL of 1X TBE electrophoresis buffer and heat until the agarose is completely melted. Place the melted agarose solution at room temperature at around 50 ℃ and add 5μl of Safe Green Stain. Shake well.

2.2. Pour glue: slowly pour the prepared agarose gel into the glue making tray to avoid bubbles. Place the sampling comb vertically on one end of the electrophoresis gel film, about 1mm away from the bottom of the tray. Try to maintain stability and avoid shaking when placing.

2.3. Glue placement: Wait for about 30 minutes for the gel to fully solidify, then slowly and vertically pull out the sample comb, without exerting too much force. (Appropriate extension of gel time in summer)

2.4. Put the agarose gel into the electrophoresis tank and add the electrophoresis buffer so that the level of the electrophoresis buffer is about 1-2 mm higher than the surface of the gel.

2.5. Add the DNA sample of mixed bromophenol blue indicator (1μl bromophenol blue mixed with 2μl DNA sample) into the sampling well.

2.6. Cover the electrophoresis tank cover, turn on the power, and 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).

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).

2.8. Observe the results with a 302 nm excited UV gel imaging system.

***Note: This method requires relatively less dye for dyeing. Dyes can be directly heated in a microwave when added to the glue, and the prepared glue solution can be stored at room temperature until used up.**

Reference for optimizing electrophoresis conditions:

Because EB is inserted into DNA to form a whole molecule, it is not easy to encounter migration/dispersion problems, while the Safe Green Stain of large molecules is non covalently bound to DNA through electrostatic attraction, making it easy for band migration to occur outside of DNA, especially large fragments of DNA!

1. Given the high sensitivity of Safe Green Stain, it is recommended to reduce the amount of DNA sample loaded. The optimal loading amount for DNA samples is~100ng/lane (conventional 8-lane small gel well).
2. The concentration of some domestically produced DNA markers is too high. Dilute twice before use! At present, some domestic markers are enzyme cut mixed fragments developed based on EB dye. Please use post staining method.
3. Replace the electrophoresis buffer, the newly configured electrophoresis solution works well! Replacing TAE with TBE buffer is more effective!
4. The voltage should not be too high during electrophoresis, generally not exceeding 130V. Compared with EB, Safe Green Stain has a lower electrophoresis voltage and longer gel running time.
5. Dyes can be stored in a dark place at room temperature or 4 ℃; If there is precipitation, heat the dye to 40-50 ℃ and shake it thoroughly to dissolve without affecting its use.
6. Due to the good thermal stability of Safe Green Stain, it can be directly added to a hot agarose solution and thoroughly shaken and mixed. Safe Green Stain can also be added to agarose powder and electrophoresis buffer, and then heated in microwave oven to prepare agarose gel.
7. Individual customers use 3X dye to mix with samples, and then sample into agarose gel. This sampling method is not recommended!
8. If you always see band dispersion or unsatisfactory separation, it is recommended to use post electrophoresis bubble staining to stain the gel.

This gel dyeing method (pre dyeing method) is not suitable for prefabricated polyacrylamide gel. For polyacrylamide gel, please use the foam dyeing method (post dyeing method).

Bubble dyeing method (post dyeing method)

Note: The banding bending and dragging phenomenon in bubble dyeing is related to the quality of samples and the concentration of gel, not the problem of dyes! The concentration of agarose gel was increased by 0.2% -0.3% by soaking than by pre staining.

1. Perform electrophoresis according to the conventional methods mentioned above. We strongly recommend the bubble staining method for high concentration DNA samples such as gel recovery!
2. Dilute the Safe Green Stain 10000 x storage solution by approximately 10000 times to a 0.1M NaCl solution to prepare a 3 x staining solution. (For example, add 15μl of Safe Green Stain 10000 x original solution to 50mL of 0.1M NaCl solution).
3. Carefully put gel into a suitable container (such as polypropylene container) and slowly add enough 3 × dye solution to immerse gel. The optimal staining time is slightly different according to the thickness of gel and the concentration of agarose. For gel containing 1%, the dyeing time is about 30min.
4. Observe the results with the 302 nm excited ultraviolet gel imaging system.

Note: When using bubble dyeing, the amount of dye used is relatively large. 3 x Safe Green Stain is stored at room temperature in dark and can be reused for about 3 times.

The PAGE steps of nucleic acid electrophoresis:

1. Put the gel prepared by TBE into the electrophoresis tank, and clamp the edge with a clamp.
2. Fill the buffer tank with 5 × TBE of the same batch of gel solution. Use a syringe to remove bubbles from the bottom of the gel.
3. Use a syringe to aspirate 1 x 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.
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.
5. Electrophoresis to standard reference dye migration to the desired position (usually electrophoresis until xylene is completely removed, and bromophenol blue stops at a distance of 2-3cm from the bottom edge). Turn off the power, unplug the plug, and discard the electrophoresis solution from the electrophoresis tank.
6. Take down the gel and put it into a dyeing dish. Add 1X buffer solution of 1X Safe Green Stain to shake and dye it for 30-60 minutes, and then place it for UV detection.

Note: PAGE cannot be pre dyed or spot dyed; The color can only be developed by bubble dyeing. Because polyacrylamide is relatively dense, the dye is not easy to penetrate, and the color development effect is not as good as that of agarose gel.

Special reminder: If using a UV imager, please select Safe Green Stain; If using a laser imager or observing under visible light, please select Safe Green Stain.