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Sybr Green I

Product Number: ST02

Description

Sybr Green I and Sybr Green II nucleic acid colorants are new generation fluorescent nucleic acid gel colorants designed to replace the highly toxic ethidium bromide (EtBr). Sybr Green I is non-toxic and more sensitive than EtBr. Gel can be visualized under ultraviolet or visible light.

Features

1. Safety: Sybr Green is nontoxic and noncarcinogenic.
2. Ultra-sensitivity: It allows the visualization of as little as 20pg dsDNA, around 5-10 times more sensitive than EtBr under UV and 8-20 times more sensitive than EtBr in Visible Light.
3. Convenience: NO need to rinse or wash gels. Add stain before load samples. Visualize gels under UV or Visible Light to avoid UV damage on DNA/RNA.
4. Wide range: suitable for agrose gel or PAGE.
5. No impact for the next experiments such as RT, PCR, enzyme digestion, and ligation.
6. Strong signal and no background
7. Low cost: 1 ml Sybr Green I Nucleic Acid Stain is sufficient to load 10,000 samples.

Protocol

1. Stain nucleic acid in electrophoresis (add stain in the gel)
   - 1.1. Make gels: Add 1μl Sybr Green I Nucleic Acid Stain per 100ml gel when cool down to 50℃. Usually, 1ml Sybr Green I Nucleic Acid Stain is sufficient for making 100 gels (100ml per gel) and one gel is enough to load 100 samples. So, 1ml Sybr Green I Nucleic Acid Stain is enough to load 5000 samples.
   - 1.2. Running the gels according to the routine method.
   - 1.3. Visualize gels under UV or Visible Light. The Visible light is much better to avoid UV damage on DNA/RNA.

   Note:Exact molecular weight can be measured by this method, also 1mL Sybr Green can be used to load 50000samples (50 samples per 100mL gel).
2. Stain nucleic acid before electrophoresis (add stain in the loading buffer)
   - 2.1. Prepare working solution: Dilute 10μl Sybr Green I Stain with 1ml running buffer TBE or TAE. This solution is stable up to one month at 4℃
   - 2.2. Make gels: based on the routine method. Do not add any DNA/RNA stain in the gel.
   - 2.3. Stain Nucleic Acid: Add 1μl Sybr Green I Nucleic Acid Stain working solution to 10μ mixture of sample and loading buffer, let it stay at RT for 3-5min for stain binding to nucleic acid completely. Normally, 1μl working solution is enough for one sample loading, and 1ml Sybr Green I Nucleic Acid Stain is enough to load 10,000 samples.
   - 2.4. Stain markers: Mix 5μL Marker and 1μL Sybr Green I Nucleic Acid Stain working solution thoroughly, let it stay at RT for 5min to let Sybr Green I Stain and DNA/RNA binding completely.
   - 2.5. Load samples and run gels.
   - 2.6. Visualize gels in UV or Visible Light to avoid UV damage on DNA/RNA.

   Noe:Usually 1μL stain is enough to stain one sample. That is 1mL Sybr Green is enough to load 100000 samples. However, big fragments (>2Kb) will move slowly when bind to the stain. So please stain in DNA after electrophoresis or add stain in gels to measure molecular weight exactly.
3. Stain Nucleic Acid after electrophoresis (add stain in gel staining solution)
   - 3.1. Make gels: Do not add any nucleic acid stain when make gels.
   - 3.2. Prepare Sybr Green I Nucleic Acid Staining solution: dilute Sybr Green I Nucleic Acid Stain with TAE or TBE on ratio

1:10000. Stain gels in the dark for 10-30min. Staining time depends on gel concentration and thickness. PAGE can be stained directly on the glass. Let staining solution cover PAGE gels for 30min. Please use glassware to store staining solution or silicified glassware because stain will absorb on the glass.

3.3. Visualize gels in UV or Visible Light to avoid UV damage on DNA/RNA.

Note:Exact molecular weight can be measured by this method but the dyes are used much more in this way.

4. Stain Nucleic Acid after electrophoresis (add stain in gel staining solution)

4.1. Combine protocol 1 and protocol 2. It is the most sensitive method in all protocols to detect nucleic acids.

5. Sybr Green II Staining RNA Following Electrophoresis

5.1. Perform electrophoresis on nondenaturing gels or on denatur-ing polyacrylamide/urea or agarose/formaldehyde gels according to standard techniques. SYBR Green II RNA gel stain has not been tested with other gel matrices.

5.2. Dilute the stock SYBR Green II RNA gel stain. For non-denaturing gels and denaturing polyacrylamide/urea gels, we recommend a 1:10,000 dilution in TBE (89mM Tris base,89mM boric acid, 1mM EDTA, pH8). For denaturing agarose/formaldehyde gels, we recommend a 1:5000 dilution in TBE. Staining with SYBR Green II reagent is pH sensitive.For opti-mal sensitivity, verify that the pH of the staining solution at the temperature used for staining is between 7.5 and 8.0 (preferably pH 8.0).

5.3. Place the gel in a staining container, such as a Petri dish or the top of a pipet-tip box. Add enough staining solution to cover the gel. Protect the staining container from light by covering it with aluminum foil or placing it in the dark. There is no need to wash urea or formaldehyde out of gels prior to staining.

5.4. Agitate the gel gently at room temperature. The optimal staining time is typically 10–40 minutes for polyacrylamide gels and 20–40 minutes for agarose gels. The staining time may vary depending on the thickness of the gel and the percentage of aga-rose or polyacrylamide. No destaining is required. The staining solution may be stored in the dark (preferably refrigerated) and reused three to four times.

5.5. Illuminate the stained gel using 300 nm ultraviolet transillu-mination, or for greater sensitivity, 254 nm epi-illumination.

Note

1. Do not run gels over 2h. Or smeared bands appeared because Sybr Green I Stain will dissociate from DNA/RNA.
2. Sybr Green I Stain can dissociate from nucleic acids in ethanol.
3. Please stain nucleic acid in the gel or after electrophoresis to check the exact molecular weight of fragments when compared with molecular weight markers.
4. Please stain DNA with DNA Stain and RNA with RNA Stain.
5. Please use EP tubes and other plastic wares in Sybr Green I Stain storage, dilution, and staining. Sybr Green I Stain can bind to glassware.