Sybr Green (10000X) Nucleic Acid Stains

Product Description:

Sybr Green I and Sybr Green II Nucleic Acid Stains are produced by Dr.Chimin Du, are a kind of novel generation of fluorescent nucleic acid gel stains designed to replace the highly toxic ethidium bromide (EtBr). Sybr Green I is nontoxic and more sensitive than EtBr. Gels can be visualized under UV or Visible Light.

Features of Sybre Green Nucleic Acid Stains

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

Directions for the Agrose Gels stained by Sybr Green

Protocol 1: Pre-cast Protocol (Add dye in the gel)

1.1 Prepare molten agarose gel solution using your standard protocol.
1.2 Add 1~3μl Sybr Green Nucleic Acid Stain per 50ml gel when the gel cool down to 50℃and mix thoroughly.
1.3 Cast the gel and allow it to solidify. Any leftover gel solution may be stored and reheated later for additional gel casting.
1.4 Load samples and run the gels using your standard protocol.
1.5 DNA stained with SYBR Green I stain can be readily visualized using a UV or blue-light sources (emit at 450, 473, 488, or 532 nm). Image the stained gel with the transilluminator and photograph the gel using.

Protocol 2: Post-staining Protocol (Stain Nucleic Acid after electrophoresis by adding dye in the gel stain solution)

2.1 Make gels: Do not add any nucleic acid stain when make gels.
2.2 Run gels as usual according to your standard protocol.
2.3 Prepare Sybr Green Nucleic Acid Staining solution: Dilute Sybr Green Nucleic Acid Stain with TAE or TBE (TBE (89 mM Tris base, 89 mM boric acid, 1 mM EDTA, pH 8) and TAE (40 mM Trisacetate,1 mM EDTA, pH 8) 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.
2.4 Visualize gels a UV or blue-light sources.
2.5 Exact molecular weight can be measured by this method but the dyes are used much more in this way.

Protocol 3: Stain nucleic acid before electrophoresis (add dye in the loading buffer)

3.1. Prepare working solution: Dilute 10 μl Sybr Green Stain with 1ml running buffer TBE or TAE. This solution is stable up to one month at 4℃
3.2. Make gels: based on the routine method. Do not add any DNA/RNA stain in the gel.
3.3. 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.
3.4. 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.
3.5. Load samples and run gels.
3.6. Visualize gels in UV or Visible Light to avoid UV damage on DNA/RNA.

*The big DNA 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.

Notes :

1. Do not run gels over 2 hrs. 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 use EP tubes and other plastic wares in Sybr Green I Stain storage, dilution, and staining. Sybr Green I Stain can bind to glassware.

Sybr Green I（10000X）核酸染料

本品用 DMSO 溶解，因为 DMSO 溶点是 18.3℃，使用前请放置到室温充分溶解。

Sybr Green I 核酸染料特点：

1. 无毒性：花菁染料，无致癌毒性。
2. 高灵敏：紫外凝胶透射仪下灵敏度高 EB 染色法 5–10 倍，可见光透射仪下的灵敏度比 EB 染色法高20~30 倍。
3. 信噪比高：样品荧光信号强，无背景信号。
4. 操作简单：无须脱色或冲洗，即可用紫外凝胶透射仪观察或可见光透射仪观察。
5. 适用范围广：可适用于多种电泳分析，如琼脂糖凝胶电泳和 PAGE 凝胶电泳。
6. 使用方便：不影响其它修饰酶作用(如：Taq 酶、内切酶、T4 连接酶、反转录酶等)。

Sybr Green I 核酸染料使用方法：

1.胶染法(用法同EB)(推荐方法，见图1)

1) 制胶时加入 Sybr Green I 核酸染料。冷却胶到 50℃左右，每 100ml 胶中加入1–3μl Sybr Green I 核酸染料（见图 1）。
2) 按照常规方法进行电泳即可。
3) 用紫外凝胶透射仪或可见光透射仪观测。蓝光可透过玻璃， 观测聚丙烯酰胺凝胶时，可直接将托有凝胶的玻璃平皿放入可见光透射仪内观测。

*注：此方法染色能准确确定片段分子量且用量较少。1ml 染料可以做 1000 块10 ml 胶，每块胶点 50 个样，可做 50000 次。

2.点染法(见图3)

1) 该方法适于琼脂糖凝胶电泳和PAGE凝胶电泳。
2) 工作液的配制：用电泳缓冲液将10000×的Sybr Green I 稀释100倍，即为Sybr Green I 工作液。Sybr Green I 工作液可以置2～8℃保存一个月以上，浓缩液在–20℃保存半年。
3) 制胶：按常规方法制胶，不含任何染料。
4) 样品染色：向分析样品中加入Sybr Green I工作液和载样缓冲液，室温放置10分钟，使Sybr Green I与样品中DNA充分结合。Sybr Green I 工作液加入量为总上样量的1/5～1/10。
5) DNA Marker染色：将5μL DNA Marker、5μL DNA Marker稀释液和1μLSybr Green I 工作液混匀，室温放置5分钟，使Sybr Green I 与DNA充分结合。
6) 上样、电泳：按常规操作。用紫外凝胶透射仪或可见光透射仪观测。蓝光可透过玻璃， 观测聚丙烯酰胺凝胶时，可直接将托有凝胶的玻璃平皿放入可见光透射仪内观测。

*注：用点染法染色时，灵敏度最高，染料用量最少。通常点一个样加入 1μL 即 可，可以使用 10000 次，但大片段稍有滞后现象，如果需要更准确确定分子量(与 Marker 对比)，建议使用胶染法。

3.泡染法

1) 按照常规方法进行制胶，其中不含任何染料。
2) 用 pH 7.0 – 8.5 的缓冲液(如：TAE, TBE)，按照 1∶1000 的比例稀释 Sybr Green I
3) 核酸染料，混匀，制成染色溶液。
4) 将染色溶液倒入合适的聚丙烯容器中，放入凝胶，用铝箔等盖住容器使染料避光。 室温振荡染色 10–30 分钟，染色时间因凝胶浓度和厚度而定。聚丙烯酰胺凝胶直 接在玻璃平皿上染色，将配好的稀释溶液轻轻地倒在胶板上，让稀释液均匀地覆 盖整个胶板，并染色 30 分钟。玻璃平皿必须预先经过硅烷化溶液处理（避免染料 吸附在玻璃表面上）。
5) 用紫外凝胶透射仪或可见光透射仪观测。蓝光可透过玻璃， 观测聚丙烯酰胺凝胶时，可直接将托有凝胶的玻璃平皿放入可见光透射仪内观测。

*注：用泡染方法染色时，可以精确确定核酸片段分子量。但染料用量是三种方法中用量最大的。

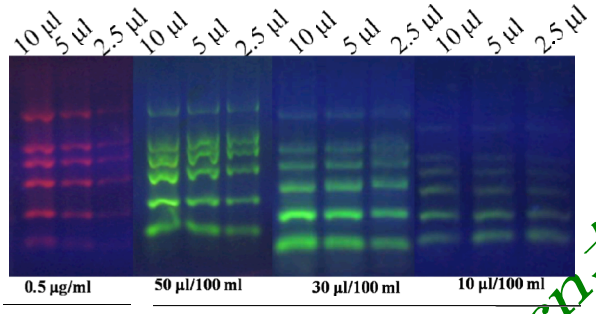
几种染色方法特点比较:

染色方法 / 特点	灵敏度	染料用量	确定片段分子量精确度
胶染法	较高	较少	较高
点染法	很高	最少	大片段稍有滞后
泡染法	较高	最多	最高
点染+胶染法	最高	较多	大片段稍有滞后

Sybr Green I 核酸染料使用注意事项:

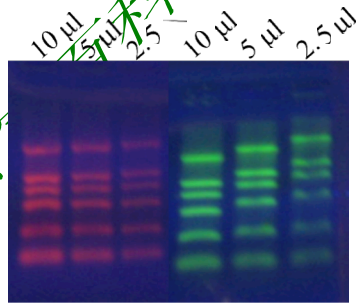
1. Sybr Green核酸染料样品点染方法中，电泳不要超过 2 小时，以免核酸染料从 DNA/RNA 上分离出来，产生弥散状条带。
2. 用点染方法染色时，条带稍有滞后现象，如果需要确定片段精确分子量（和 Marker 对比），建议用胶染法和泡染法。
3. 常规用酒精沉淀核酸过程中，Sybr Green I 核酸染料可以全部从核酸上去掉。
4. DNA电泳请选择 Sybr Green I 染料，RNA 电泳请选择 Sybr Green II染料，两种染料不通用。
5. Sybr Green I 核酸染料对玻璃和非聚丙烯材料具有一定亲合力。建议在稀释、贮存、染 色等使用过程用聚丙烯类容器。
6. 可以加Orange Red 作为标记. pH值在7.5–8.3之间,不要微波加热,加入热胶的温度低于50度

Sybr Green I 核酸染料不同使用方法电泳图谱:



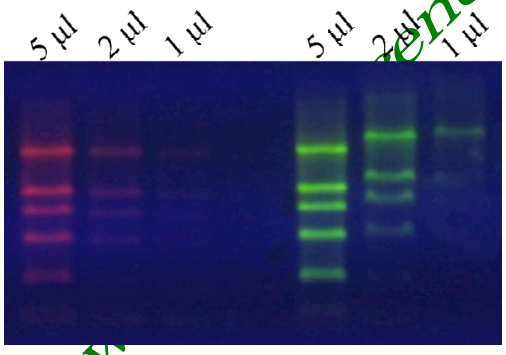
EB Sybr Green I DNA Stain

Fig.1 胶染法 Fig.1 DNA marker 2000 10, 5, 2.5 per lane



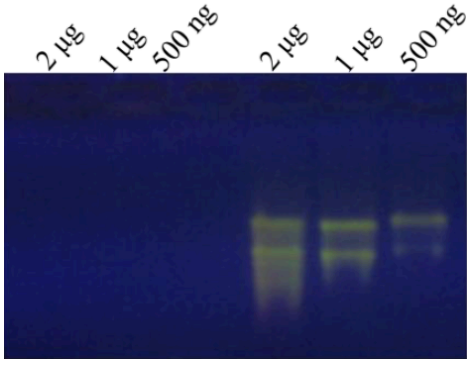
EB Sybr Green I

Fig.2 胶染+点染 Fig.2 DNA Marker 2000 incubate at RT for 3~5min



EB Sybr Green I DNA Stain

Fig3. 点染法 Fig.3 Volume ration of Dye/DNA marker 2000=1:10 incubate at RT for 3~5min then load 5,2,1uL per lane.



EB Sybr Green II RNA Stain

Fig.4 Incubate at RT for 3~5min then load 0.5,1,1ug per lane
Fig4. Sybr Green II 点染(RNA)