SafeRed®/DuGreen® 核酸染料（10000× 水溶液）

SafeRed®/DuGreen®核酸染料特点

1. 带形清晰整齐：第三代SafeRed®和DuGreen®完全克服了原装国内外类似染料分不开大片段DNA的缺点，条带清晰整齐美观。
2. 安全无毒：独特油性大分子使其不能穿透细胞膜进入细胞， Ames-test实验表明，该染料的没有EB类似的诱变性。
3. 迁移率好：EB小分子很快跑出胶外，所以EB容易导致小DNA片段看不清，我们的大分子SafeRed完全克服这一点。
4. 定量准确：适用于核酸分子大小的确定和定量，EB对小DNA片段定量不准确。
5. 染色均匀：整个凝胶负极端和正极端的亮度一样。EB会导致胶的整体背景稍微高些，经常出现阴 阳背景（胶的背景一部分亮一部分暗）；EB长时间、长距离的电泳，EB信号强度会相应下降。我们的大分子SafeRed完全克服这一点。
6. 灵敏度高：适用于各种大小片段的电泳染色，对核酸迁移的影响小于SYBR Green I。
7. 稳定性高：耐热，可加在缓冲液里，100℃溶解凝胶，防止染色剂没充分混匀。适用于微波或其它加热方法制备琼脂糖凝胶。
8. 耐光性强：实验室的日常光线照射环境下可以常温放置24个月。
9. 信噪比好：样品荧光信号强，背景信号低，荧光亮度是EB的十倍以上，肉眼可观测到亮度明显比EB强。
10. 操作简单：与EB用法完全一样，在预制胶和电泳过程中染料不降解；而电泳后染色过程也只需30 分钟且无需脱色或冲洗。
11. 适用范围广：适用于琼脂糖凝胶或聚丙烯酰胺凝胶电泳；可用于 dsDNA、ssDNA 或 RNA 染色。
12. 完美兼容：SafeRed兼容所有的紫外凝胶透射仪；DuGreen兼容所有的蓝光仪和可见光仪器。Safe与EB有相近的光谱特性，无需改变滤光片及观察装置：标准的EB滤光片或SYBR滤光片都适用，使用和EB相同紫外凝胶透射仪，在300nm紫外光附近可得到最佳激发。

产品包装： SafeRed® 10000x Nucleic Acid Dye 500uL/支； DuGreen® 10000x Nucleic Acid Dye 500uL/支

储存条件： 室温或4℃避光可保存24个月。

操作步骤：

一．胶染法（预染法，用法EB完全相同）

制胶时加入SafeRed 核酸染料(染料灵敏，每100mL 琼脂糖溶液中加入10μL SafeRed 原装液即可)。 按常规方法电泳。

1. 实验室材料和试剂：

（1） 实验样品：质粒 DNA，DNA marker（国产的 DNA marker 浓度太高，至少稀释 2～3 倍后使用）

（2） TBE 缓冲液配置：10X TBE 电泳缓冲液[Tris 107.8146g (890mM),硼酸 55.0287g(890mM)，EDTA 5.845g(20mM),加 NaOH 约 4g 调节 pH=8.3；定容 1000mL]；用 ddH_2O 稀释 10 倍配制 1X TBE 电泳缓冲液。

（3） TAE 缓冲液配置：50X TAE 电泳缓冲液[Tris 242g (2M),EDTA 37.2g(100mM),加醋酸约 57ml 调节 pH=8.5；定容 1000mL]；用 ddH_2O 稀释 50 倍配制 1X TAE 电泳缓冲液。

（4） 溴酚蓝指示剂，1%的西班牙琼脂糖凝胶

（5） 仪器：电泳仪（130v），移液器(0.5~10ul)，凝胶成像仪

2. 实验步骤：

（1） 制胶：将 0.5g 琼脂糖溶于 50mL 1X TBE 电泳缓冲液中，加热至琼脂糖完全融化。将融好的琼脂糖溶液室温放置 50℃左右加入 5uL 的 SafeRed 凝胶电泳染料，摇匀。

（2） 倒胶：将制好的琼脂糖凝胶缓慢倒于制胶托盘内，避免产生气泡。将点样梳子垂直置于电泳胶膜的一端，距离托盘底部约 1mm。放置时尽量保持平稳，切勿晃动。

（3） 置胶：待约 30 分钟左右胶体充分凝固后，缓慢垂直向上拔起点样梳子，切勿用力过猛。（夏季适当延长凝胶时间）

（4）将琼脂糖凝胶放入电泳槽内，加入电泳缓冲液，使电泳缓冲液液面高于凝胶面约 1~2mm。

（5）将混合溴酚蓝指示剂的 DNA 样本（1ul 溴酚蓝与 2ul DNA 标本混合）加入到点样孔内。

（6）盖上电泳槽盖，开启电源，使 DNA 从负极移向正极恒压电泳(电压恒定在 120~130v 之间，一般可选择 130V)。

（7）当 DNA 条带距离点样孔约 1~2cm 后关闭电源，(约 30～40 分钟)取出凝胶。

（8）用 302nm 激发的 UV 凝胶成像系统观察结果。

***注：此方法染色染料用量相对较少。染料加入胶中可直接使用微波炉加热，制好的胶溶液可以在室温下保存直至用完。**

优化电泳条件参考事项：

因为EB是插入DNA内部变成一个整体分子，所以不容易出现迁移/弥散的问题，而大分子的SafeRed与DNA是通过静电吸引非共价结合的，在DNA外面就容易出现条带迁移，特别是大片段DNA！

1） 鉴于 SafeRed 的高灵敏性，建议减少 DNA 的上样量。DNA 样品最佳上样量为～100ng/泳道(常规 8 泳道小胶孔)。
2） 部分国产的DNA marker浓度太高，至少稀释一倍后使用！目前国产的部分marker是基于EB染料开发的酶切的混合片段，请使用后染法。
3） 更换电泳缓冲液，新配置的电泳液效果好！用 TBE 缓冲液代替 TAE 效果更好！
4） 电泳时电压不宜过高，一般不要超过130V。与EB相比，SafeRed电泳电压要低一些，跑胶的时间长一些。
5） 染料在室温或 4℃下避光储存即可；若有沉淀，将染料加热至 40~50℃并充分振荡溶解，不影响使用。
6） 由于SafeRed具有良好的热稳定性，可以在热的琼脂糖溶液中直接添加后充分振荡混匀。SafeRed也可以加到琼脂糖粉末和电泳缓冲液中，然后用微波炉加热以制备琼脂糖凝胶。
7） 个别客户用3X染料和样品混合后，点样到琼脂糖凝胶中，不推荐这种点样
8） 如果总是看到条带弥散或分离不理想，建议使用电泳后泡染法染胶。

***此胶染法(预染法)不适合预制聚丙烯酰胺凝胶，对于聚丙烯酰胺凝胶请使用泡染法(后染法)。**

二．泡染法(后染法)

（1）按照以上常规方法进行电泳。 **用于胶回收等高浓度DNA样品强烈推荐泡染法！**

（2）将SafeRed® 10,000×储液稀释约3,300倍到0.1M NaCl溶液中制成3×染色液。(例如将15μL SafeRed® 10,000×原装液加入到50mL 0.1M NaCl溶液中)。

（3）将凝胶小心地放入合适的容器中（如聚丙烯容器中），缓慢加入足量的3× 染色液浸没凝胶。室温振荡染色30min左右，最佳染色时间根据凝胶厚度以及琼脂糖浓度不同而略有不同。对于含1%的凝胶，染色时间约30min。

（4）用 302nm 激发的紫外凝胶成像系统观察结果。

***注意事项：用泡染法染色时，染料用量较多。3× SafeRed®染色液室温避光保存，可重复使用3次左右。**

三．核酸电泳的PAGE步骤：

1）将TBE制备的凝胶放入电泳槽中，用夹子夹住边缘。

2）用配置凝胶溶液同一批次的5×TBE灌满缓冲液槽。用注射器排除凝胶底部的气泡。

3）用注射器吸取1×TBE冲洗加样孔。将DNA样品和适量的6×凝胶上样缓冲液混合，用微量移液管加入加样孔。

4）将电极与电源相连（正极接下槽），打开电源一般90V；1～8V/cm。进行电泳9h。

5）电泳至标准参照染料迁移至所需位置（一般是电泳到二甲苯完全迁出，溴酚蓝距底边2～3cm停止）。关闭电源，拔掉插头，弃去电泳槽中的电泳液。

6）将凝胶取下来放入，染色皿中，加3X SafeRed的1X缓冲液中的振荡染色30–60分，放置在紫外检测即可。

***注意事项：与琼酯糖凝胶不同，不能用预染或点染的方法；只能用泡染的方法显色，由于聚丙烯酰胺比较致密，染料不容易深入，显色效果没有琼酯糖凝胶好。**

特别提醒： 如果用的是紫外成像仪，请选择SafeRed；如果使用激光成像仪或在可见光下观测，请选择DuGreen。

SafeRed® Nucleic Acid Gel Stain

Product Description

SafeRed® Nucleic Acid Gel Stain is the third generation nonmutagenic fluorescent nucleic acid gel stain to replace the highly toxic traditional ethidium bromide (EtBr) for staining dsDNA, ssDNA or RNA in agarose gels or polyacrylamide gels. The third part's Ames test by WuXi Pharmacy shows that SafeRed and DuGreen are nonmutagenic for gel staining. DuGreen and SafeRed Nucleic Acid Gel Stain are highly sensitive than EtBr either as precast gel stains or post gel stains.

We can directly replace EB with SafeRed® without changing the existing imaging system since SafeRed® and EB have the similar spectra. SafeRed® can also be used to stain dsDNA, ssDNA or RNA in polyacrylamide gel via post gel staining. Gel staining with SafeRed® is compatible with downstream applications such as gel extraction and cloning. SafeRed® is efficiently removed from DNA by phenol/chloroform extraction and ethanol precipitation.

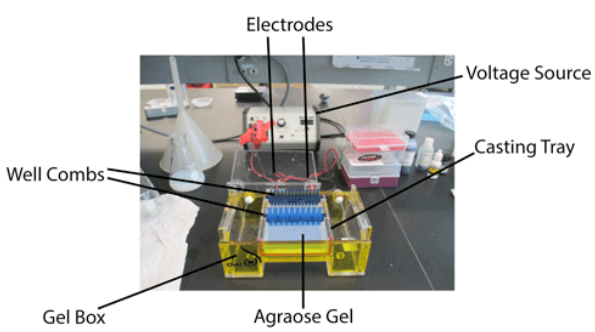
Features

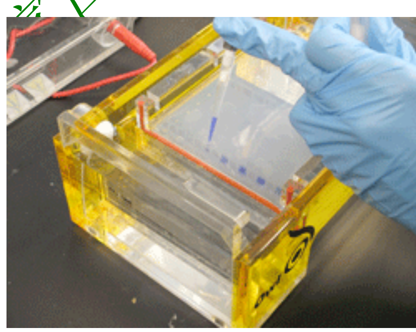
- Safety: Nonmutagenic and noncytotoxic
- Easy disposal: Safe to dispose in the drain
- Compatibility: Spectrally compatible with all the existing instruments
- Sensitivity: Higher signal but lower background
- Stability: can be stored at RT and microwavable

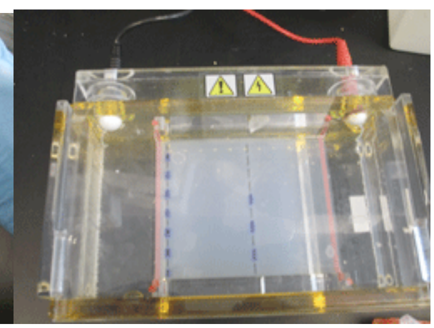
Materials needed:

Agarose, TAE Buffer, 6X Sample Loading Buffer, DNA ladder standard, Electrophoresis chamber, Power supply, Gel casting tray and combs, Staining tray, Gloves, Pipette and tips

Precast Protocol for Agarose Gel Electrophoresis







Pouring a Standard 1% Agarose Gel:

1 Pour 1g of Agarose powder and 100mL of 1xTAE or 1xTBE into a glass flask.
   ***Note:** Agarose gels are commonly used in concentrations of 0.7% to 2% depending on the size of DNA bands.*

2 Melt the agarose in a microwave for 1-3min (until the agarose is completely dissolved and the solution becomes clear, the solution is completely clear and no small floating particles are visible.)
   ***Note:** Caution HOT! Be careful!* Do not let the solution boil for long periods as it may boil out of the flask.

3 Let agarose solution cool down to about 50-55°C, Add 10uL SafeRed per 100mL gel, gently stirring the gel solution to mix thoroughly.

4 Seal horizontal gel apparatus. Pour molten agarose onto gel plate to a depth of 4~8 mm. Insert a comb until its base is 1 mm from the base of the gel. Allow cooling.
   ***Note:** Pour slowly to avoid bubbles, which will disrupt the gel. Any bubbles can be pushed away from the well comb or towards the sides/edges of the gel with a pipette tip.*

5 Place newly poured gel at 4°C for 10-15 minutes OR let sit at room temperature for 20-30 minutes, until it has completely solidified. Remove comb and submerge in 4X TAE buffer

Loading Samples and Running an Agarose Gel:

1 Once solidified, place the agarose gel into the gel box.
2 Add 1/5 volume 6X Loading buffer to sample.
__Note:__ Loading buffer serves two purposes: 1) it provides a visible dye that helps with gel loading and will also allows you to gauge how far the gel has run while you are running your gel; and 2) it contains a high % glycerol, so after adding it your sample is heavier than water and will settle to the bottom of the gel well, instead of diffusing in the buffer.
3 Fill gel box with 1xTAE (or TBE) until the gel is covered.
4 Carefully Pipette 5 μl of the DNA ladder standard into the first lane of the gel.
__Note:__ When loading the sample in the well, maintain positive pressure on the sample to prevent bubbles or buffer from entering the tip. Place the very top of the tip of the pipette into the buffer just above the well. Very slowly and steadily push the sample out and watch as the sample fills the well. After all samples are unloaded, push the pipettor to the second stop and carefully raising the pipette straight out of the buffer.
5 Carefully pipette 5 μl of each sample/Sample Loading Buffer mixture into other separate wells in the gel.
6 Run the gel at 80-150V until Bromophenol Blue is near the end of the gel. This dye runs at ~800b.
__Note:__ Black is negative, red is positive. (The DNA is negatively charged and will run towards the positive electrode.) __Note:__ A typical run time is about 0.5-1 hour, depending on the gel concentration and voltage.
7 Turn OFF power, Disconnect the electrodes from the power source, and then carefully remove the gel from the gel box.
8 Visualize or Image the stained gel with a standard transilluminator (302 or 312nm), and photograph the gel using an ethidium bromide filter.

Post-Staining Protocol

1. Run Agarose Gel Electrophoresis according to hereinbefore.
2. Dilute 10000X SafeRed stock solution to 3X staining solution in H_2O with 0.1M Nacl(e.g, add 15ul of SafeRed® 10,000X stock reagent and 5ml 1M Nacl to 45ml H_2O). Note: including 0.1M NaCl in the staining solution enhances sensitivity, but may promote dye precipitation if the gel stain is reused. (This solution can be reused at least 2~3 times protected from light).
3. Using gloves, carefully remove the gel from the casting tray and place into the staining dish, add 3X SafeRed staining solution to submerge the gel.
4. Agitate the gel gently at room temperature for~30 minutes and the amount of the stain may depend on the thickness of the gel and the percentage of the agarose with gently shaking.
5. Rinsing the gel with water can reduce the background.
6. Visualize or Image the stained gel with a standard transilluminator (302 or 312nm), and photograph the gel using an ethidium bromide filter.

Tips and FAQ

◆ **How do you get better resolution of bands?**
A few simple ways to increase the resolution (crispness) of your DNA bands include: a) running the gel at a lower voltage for a longer period of time; b) using a wider gel comb; or c) loading less DNA into well.

◆ **How do you get better separation of bands?**
If you have similarly sized bands that are running too close together you can adjust the agarose percentage of the gel to get better separation. A higher percentage agarose gel will help resolve smaller bands from each other, and a lower percentage gel will help separate larger bands.

◆ **10% Rule:**
For each sample you want to load on a gel, make 10% more volume than needed because several microliters can be lost in pipetting. For example, if you want to load 1.0μg in 10μL, make 1.1μg in 11μL.

◆ **Diffused strip or smeared DNA band(s).**
SafeRed and DuGreen cannot go into the DNA double helix structure like EB because their big molecular weight is more than 1000. Please try the following methods to reduce the smeared DNA band(s) or diffused strip:1) Load half of your DNA sample and DNA marker; 2) Pour a lower percentage agarose gel for better resolution of large fragments; 3) Using the fresh TAE buffer or TBE buffer instead of TAE. 4) Please run the gel stain by the post-staining protocol.