M5 Gelred Plus 核酸染料（更清晰的 EB 替代品）使用说明书

产品名称	单位	货号
M5 Gelred Plus 核酸染料(10000X)	500μl	MF079-plus-01
M5 Gelred Plus 核酸染料(10000X)	5x500μl	MF079-plus-05

【储存条件】
2-8℃（避免太阳光直射）。

【产品组分】

	MF079-plus-01	MF079-plus-05
M5 Gelred Plus 核酸染料(10000X)	500μl	5x500μl

【产品简介】
M5 Gelred Plus 核酸染料(10000X)是在 Gelred 基础上优化开发的一种具有凝胶染色特性，并被设计为替换高毒性染色剂－溴化乙锭（EB）的红色荧光核酸染色剂，比 Gelred 具有更高的清晰度和灵敏性。 因为 Gelred Plus 与 EB 有着相同的光谱特性，可以在不改变任何成像系统的情况下用来替换 EB。如果使用的是 SYBR（如 SYBR Green 1/SYBR Gold)染色剂，并使用紫外透射器(UV transilluminator）来观察凝胶，那可以使用 Gelred Plus 替换 SYBR 染色剂，不需要更换现有的 SYBR 虑光片。然而，在 488 nm 激光或类似可见光下 Plus 不能被充分地激发，如果需要，建议您使用 GelGreen 染色剂（MF147-plus），其灵敏度与 SYBR Green I 一样，但其稳定性和可靠性远胜于后者。M5 Gelred Plus 既可用于前染 (precast gel staining)，也可用于后染 (post gel staining)。通常后染比前染能够获得更灵敏的特性，并能排除染色剂在电泳过程中对核酸条带分离造成任何影响的可能性。然而，前染较后染更为简单、经济，因为前染不需要额外的着色过程，并且染料用量更少。另外，与 GelGreen 、 EvaGreen 一样，相对 EB 或 SYBR， Plus 诱导突变的能力极低。 **M5 Gelred Plus 核酸染料, 10,000X in water 为浓缩的 Gelred Plus 溶液**。用于前染时，可稀释 10,000 倍后使用；用于后染时，建议您稀释 3,300 倍后使用，见具体操作步骤。

【产品特点】
1. 安全无毒： 独特的油性大分子特点使其不能穿透细胞膜进入细胞内，艾姆斯氏试验结果也表明该染料的 诱变性远小于 EB。
2. 灵敏度高：适用于各种大小片段的电泳染色，对核酸迁移的影响较小。样品荧光信号强，背景信号低。
3. 稳定性高：适用于使用微波或其它加热方法制备琼脂糖凝胶；室温下在酸或碱缓冲液中极其稳定，耐光性强。**而且不挥发**！
4. 操作简单：在预制胶和电泳过程中不降解，可直接用可见光凝胶透射仪观察。
5. 适用范围广：可选择电泳前染色（胶染法）或电泳后染色（泡染法）；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。

【操作步骤】

一、胶染法（前染法）（用法同 EB）

1. 按常规操作，制备琼脂糖凝胶，加入浓缩的 10000X Gelred Plus， 使其在凝胶中的终浓度为 1X Gelred（比如，制备 100ml 凝胶，加入染料 10μl，可根据实际情况调整用量），轻轻摇匀，倒胶。

2. **因为非常灵敏，电泳过程中 DNA marker 上样量只需 1-2ul，而不是 EB 电泳中的 5ul，请严控 DNAmarker 上样量。**

3. 按常规方法电泳，观测结果。

二、泡染法（后染法，具体方法见背面）

1. 按照常规方法进行电泳。

2. 用 dH_2O 将 10000X Gelred Plus 浓缩液稀释约 3300 倍到 0.1M 的 NaCl 中，制成 3X 染色液。（比如，将 15μl 10000X Gelred Plus 浓缩液和 5ml 1M NaCl 加到 45ml dH_2O 中）。

3. 将凝胶小心放入合适的容器中，缓慢加入足量的 3X 染色液浸没胶。室温振荡染色约 10-30min，最佳染色时间根据凝胶厚度及琼脂糖浓度不同而略有不同。对于 3.5-10%丙烯酰胺胶，染色时间通常介于 30min 到 1 小时。然后观测结果。

【附录：后染胶标准操作流程】

核酸电泳后染胶因为污染区域小，污染操作可控，越来越得到实验室的接受和采用。

标准操作步骤（以配 100ml　1%的琼脂糖为例）：

1、 称 1g 琼脂糖，量取 100m l 1xTAE（或 1x TBE）电泳缓冲液， 依次倒入一个三角瓶中。
2、 在微波炉中化胶煮沸致琼脂糖完全融化。
3、 取出静置 5 分钟，待胶液温度降至 50 度，将胶液倒入制胶模上。
4、 20 分钟后待胶完全成型，取出放入电泳槽。
5、 将 PCR 产物或者其他 DNA 样品和上样缓冲液混合，逐一上样。
6、 电泳 20-30 分钟，根据溴酚蓝位置判断电泳到合适时间，停止电泳。
7、 将跑完电泳的胶放入含有染料的液体中，染胶 10 分钟（如果胶厚适度延长时间）。
8、 取出胶，放入扫胶仪中观察结果。

染胶液的配置：180ml dH2O 中加入 20ml 1M NaCl，再加入 10000x Gelred 浓缩液 60ul，也可以向聚合美购买即用型 1x 染胶液（货号：MF834-01，500ml）

染胶液的使用：配置好的染胶液可以重复使用很多次，直至染胶强度很低再重新配置。

GelRed Nucleic Acid Gel Stain

Description

GelRed Nucleic Acid Gel Stain is a kind of new generation of fluorescent nucleic acid gel stain designed to replace the highly toxic ethidium bromide (EtBr). The Ames test confirmed that Gelgreen are nonmutagenic at concentrations well above their working concentrations used for gel staining. Gelgreen Nucleic Acid Gel Stain is highly sensitive than EtBr either as precast gel stains or post gel stains.

GelRed and EB have virtually the same spectra, so you can directly replace EB with GelRed without changing your existing imaging system. GelRed cannot be sufficiently excited with a 488nm argon laser or similar visible light. GelRedcan also be used to stain dsDNA, ssDNA or RNA in polyacrylamide gel via post gel staining. Precast polyacrylamide gel staining with GelRed is not recommended because of relatively high background fluorescence.

GelRed Nucleic Acid Gel Stain 10,000X in DMSO is a concentrated Gelgreen solution that can be diluted 10,000 times for use in precast gel staining for ~3,300 times for use in post gel staining according to the procedures described below. One vial (0.5ml) of 10,000X solution can be used to prepare at 100 precast minigels or post-stain at least 100 minigels.

Gel staining with GelRed is compatible with downstream applications such as gel extraction and cloning. GelRed 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 existing instruments
- Sensitivity: Higher signal but lower background
- Stability: can be stored at RT and microwavable

1.Post-staining Protocol

1.1 Run gels as usual according to your standard protocol.

1.2 Dilute the GelRed 10,000X stock reagent~3,300 fold to make a 3X staining solution in H_2O with 0.1M Nacl(e.g, add 15ul of GelRed 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.

1.3 Carefully place the gel in a suitable polypropylene container. Gently add a sufficient amount of the 3X staining solution to submerge the gel.

1.4 Agitate the gel gently at room temperature for~30 minutes.

1.5 Image the stained gel with a standard transilluminator (302 or 312nm), and photograph the gel using an ethidium bromide filter.

1.6 Staining solution can be reused at least 2~3times. Store staining solution at room temperature protected from light.

2. Pre-cast Protocol

2.1 Prepare molten agarose gel solution using your standard protocol.

2.2 Dilute the GelRed 10,000X stock reagent into the molten agarose gel solution at 1:10,000 (e.g.,5ul of GelRed 10,000Xstock reagent added to 50ml of the gel solution)and mix thoroughly. Gelgreen can be added while the gel solution is still hot.

2.3 Cast the gel and allow it to solidify. Any leftover gel solution may be stored and reheated later for additional gel casting. GelRed precast gels may be stored at 4°C for later use.

2.4 Load samples and run the gels using your standard protocol.

2.5 Image the stained gel with a standard transilluminator(302 or 312nm), and photograph the gel using an Ethidium bromide filter.

Note: The pre-cast protocol is not recommended for polyacrylamide gels. Although the post-staining method is recommended, precast gels may also be tried with GelRed. However, some DNA samples, such as those derived from plasmid DNA digestion by certain restriction enzymes, may experience migration retardation or compromised resolution. Thus, both the post-stained and precast gels can be performed to determine which one may better meet your needs.

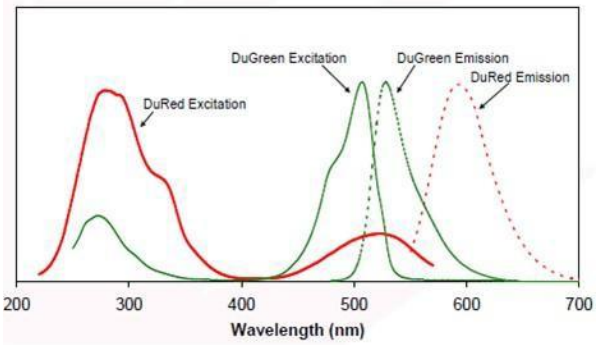

Figure 1. Normalized excitation and emission spectra of Gelgreen (green) and GelRed (red) in the presence of dsDNA in PBS buffer

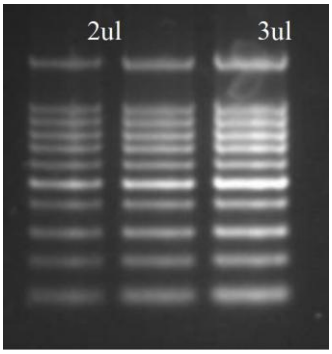

Figure 2. GelRed Pre-cast Protocol

GelRed and Gelgreen troubleshooting

1. Why am I seeing smeared or smiling DNA band(s) or discrepant DNA migration? GelRed and Gelgreen **cannot** penetrate live cell membranes then go into the DNA double helix structure like EB because they are larger molecules (big molecules than EB). They are high affinity dyes designed to be larger dyes to improve their safety. So they may affect the migration of DNA in precast gels. Specially, such as restriction digested DNA may migrate abnormally in precast gels.
Please try the following methods to reduce the smeared or smiling DNA band(s) or discrepant DNA migration:

1) **Tip #1: Load less DNA** Smearing and smiling in GelRed or Gelgreen precast gels most often caused by overloading of DNA. If you see band migration shifts or smearing and smiling, try reducing the amount of DNA loaded. The recommended loading amount for ladders and samples of known concentration is 50-200 ng/lane. For samples of unknown concentration, try loading one half or one third of the usual amount of DNA. This usually solves band migration problems.. Blown out or smeared bands can be caused by overloading. This is frequently observed with DNA ladders.
2) **Tip #2: Try the post-staining protocol** To avoid any interference the dye may have on DNA migration, we recommend using the post-staining protocol. If your application requires loading more than the recommended amount of DNA, use the post-staining protocol. While we recommend post-staining gels for 30 minutes, you may be able see bands in as little as five minutes, depending on how much DNA is present. Post-staining solutions can be reused.
3) **Tip #3:** Pour a lower percentage agarose gel for better resolution of large fragments. Higher molecular weight DNA separates better with a lower percentage gel.
4) **Tip #4:** Change the running buffer. TBE buffer has a higher buffering capacity than TAE.
5) **Tip #5:** If you see DNA migration issues or smearing after post-staining with GelRed or Gelgreen, then the problem is not caused by the nucleic acid dye. Avoid overfilling gel wells to prevent smearing of DNA down the surface of the gel.

1) 2. Why do I see weak fluorescence, decreased dye performance over time, or a film of dye remaining on the gel after post-staining?The dye may have precipitated out of solution.
2) Heat GelRed solution to 45-50°C for two minutes and vortex to dissolve.
3) Store dye at room temperature to avoid precipitation. The GelRed and Gelgreen are stable in room temperature more than one year.