GENAXXON bioscience
fon: +49(0)731 – 3608 123
fax: +49(0)731 – 3608 962
E-Mail: info@genaxxon.com

Staining Protocols

1. **In-slot staining protocol (recommended by Genaxxon)**

   a. Prepare agarose gel as usual without adding any dye.

   b. Prepare diluted GelRed™ solution (final concentration of 20X).
   Add 3µL of the 10000X GelRed™ solution to 97µL H2O resulting in a 300X GelRed™ solution.
   Add 8µL of the 300X GelRed™ solution to 100µL 6X Loading buffer plus 12µL H2O resulting in a 20X GelRed™ solution.

   Add 1µL to 2µL of the 20X GelRed® solution to 4µL sample and apply directly into the slot of the agarose gel!

   At Genaxxon this procedure show the best results in sharpness of bands and is the most economic way to use GelRed™!

2. **Staining DNA by Post Gel Staining**

   a. Run gels according to your standard protocol.

   b. Dilute the GelRed™ 10000X stock solution about 3,300 fold to prepare a 3X staining solution in water with 0.1M NaCl (e.g. add 15µL of GelRed™ stock solution and 5mL NaCl to 45mL water). While GelRed™ 1X staining solution can also be used for post gel staining, the sensitivity is generally less than with 3X staining solution (**NOTE:** inspect the 10000X vial carefully. If dye precipitation occurs, heat up or sonicate the vial).
   NOTE: Use of NaCl in the staining solution is optional. NaCl in the solution enhances the staining, but may promote dye precipitation if the staining solution is to be used repeatedly. Any staining solution to be re-used is preferably stored at room temperature in a dark place to reduce possible dye precipitation problems.

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

   d. Agitate gel gently at room temperature for about 30 minutes. Optimal staining time may vary somewhat depending on the thickness of the gel and the percentage of agarose. For polyacrylamide gels containing 3.5 – 10% acrylamide, typical staining time is 30 minutes up to 1 hour with gels of higher acrylamide content requiring longer staining time. The staining solution can be re-used at least 2-3 times. The unused staining solution can be stored at room temperature in a dark place.

   e. View the stained gel with a standard transilluminator (302nm o 312nm) and photograph the gel using an ethidium bromide filter. Similarly, a SYBR™ or GelStar(R) filter may also be used for photographing with equally good results.

3. **Staining of DNA by Precasting** GelRed™ **Gels***

   a. Prepare agarose gel solution using your standard protocol.

   b. Dilute GelRed™ 10000X stock solution into the agarose gel solution at 1:10000 (e.g. 5µL stock solution added to 50mL of gel solution). Since GelRed™ is generally thermally stable the 10000X stock solution can be added while the gel solution is still hot. Make sure that the dye is thoroughly mixed with the gel solution by swirling, stirring, or inversion.
   NOTE: inspect the 10000X vial carefully. If dye precipitation occurs, heat up or sonicate the vial).

   Alternatively the stock solution may be pre-combined with agarose powder and electrophoresis buffer of your choice followed by microwaving or other heating procedures commonly used for preparing agarose gels. GelRed™ is compatible with all commonly used electrophoresis buffers.

   c. Cast the gel and allow it to solidify. Any leftover gel solution may be stored and re-heated later for additional gel casting. Since GelRed™ is hydrolytically stable, GelRed™ precast gels may be prepared in large quantities and stored for later use. To avoid mould formation, we recommend that the precast gels be stored in a refrigerator.

   d. Load samples and run the gels using your standard protocol.
   NOTE: Use only 1/3 or less of your normal DNA-marker amount to avoid overexposure (no bands of marker visibly separated).

   e. View the stained gel with a standard transilluminator (302nm o 312nm) and photograph the gel using an ethidium bromide filter. Similarly, a SYBR™ or GelStar® filter may also be used for photographing with equally good results.
   NOTE: If you consistently see band smearing and/or poor band separation, run a gel and post-stain by following the protocol provided (point 1) to confirm if the problem is caused by the dye or other factors unrelated to the dye. If post gel staining is normal and the problem is not caused by the dye, try any of the followings:

   - Lower the amount of nucleic acid loaded.
   - Lower running voltage
   - Lower the amount of agarose in the gel
   - Run a longer gel
   - Increase the thickness of the gel
   - Increase the solidification time to ensure sharp well formation
   - Improve your loading technique or select post gel staining

*** Precasting GelRed™ gel is not suitable for acrylamide gels. Use post gel staining for acrylamide gels.**