GRRed Loading Buffer, 6X

Catalog #: **EG-1013**

GRRed nucleic acids loading buffer contains GRRed Nucleic Acid Stain, a safe and highly sensitive fluorescent dye that enables instant band visualization during or immediately after running your agarose gel. Supplied in 6X loading buffer, it forms a tight complex with the sample nucleic acids and co-migrates with nucleic acids during electrophoresis. After electrophoresis, simply place the gel on a standard UV transillumiantor to view nucleic acids bands. No post staining or destaining is required. **GRRed** provides a faster, safer, environmental friendly alternative to ethidium bromide.

Safe: GRRed is non-mutagenic in Ames tests performed by an independent testing laboratory. Your lab will have no hazardous EtBr waste: you can simply dispose the gel as regular trash.

Easy: Supplied in 6X loading buffer, simply mix loading buffer with nucleic acid sample (*e.g.,* add 2 µl of the loading buffer to 10 µl nucleic acids sample), run on gel, and visualize bands using a UV transilluminator (no post staining required).

Sensitive: GRRed is more sensitive than ethidium bromide.

Economical: No hazardous shipping and storage charges or expensive waste treatments.

Storage and Handing

- For long term storage, **GRRed** loading buffer should be aliquoted and stored at -20 ℃ protected from light. The loading buffer is stable for 1 year at -20 ℃ and 1 month at 4 ℃ or room temperature.

- **GRRed** dye can be completely removed from nucleic acids by alcohol precipitation or Qiagen QIAquick Gel Extraction.

- **Amount of DNA will affect resolution.** Use 0.1-50 ng DNA per band for samples containing one unique band, or up to 200 ng per lane for samples containing multiple bands. If you are unsure how much to use, test a range of concentrations to determine the optimal concentration for your particular sample. Excess DNA will cause poor resolution such as smearing. To obtain the best results, samples containing high concentration of salt or DNA should be diluted with water before loading to the gel.

- **Dilute samples before loading to agarose gel:** Samples containing > 50 mM NaCl, 100 mM KCl, 10 mM acetate ions, or 10 mM EDTA (i.e. certain restriction enzyme and PCR buffers) will cause loss of resolution on agarose gels. Dilute samples 2- to 20-fold as described below:

  Restriction Digests: Digest 500 ng-1 µg of DNA in 20 µl final volume. Use 1 µl (for samples >1 kb) or 2-5 µl (for samples <1 kb) and diluted with water to obtain desired concentration of salt and DNA, then mixed with **6X Loading Buffer** prior to loading on to the gel.

  PCR Samples: Use 1-5 µl of a 25 or 50 µl reaction and dilute with water to yield desired concentration of salt and DNA, then mixed with **6X Loading Buffer** prior to loading on to the gel.

Staining Protocols

Add 2 µl of the 6X loading buffer to 10 µl DNA sample and DNA marker/ladder (make sure total amount of DNA for each lane is **less** than 250 ng). Load samples and run the gel using your standard protocol. View DNA bands using a UV transilluminator.

TroubleShooting

1, Smearing, erratic (distorted) bands.

- ✓ Load less DNA (optimal DNA amount: <200 ng total DNA per lane).
- ✓ Use 0.5 X TAE or 0.5 X TBE instead of 1X TAE or 1 X TBE
- ✓ Run at lower voltage
- ✓ Do not add detergent such as SDS or Trition to your samples.
- ✓ Avoid using commercial read–to-use DNA ladder containing high concentration of salt and SDS. Consider using prestained 100 bp and 1 kb ladders from labsupplymall.com.
- ✓ Use 0.7 ~ 1.2% gel for large DNA fragments. Gel concentration >1.2% is not recommended for DNA size >3 kb.

- ✓ For separating <1 kb fragments such as PCR products, 0.7% to 1.5% gel with Lithium/Sodium Borate or TAE buffer is recommended.

- ✓ Keep running buffer fresh and cool. Warm or hot buffer will result in distorted bands.
- ✓ DNA samples with high concentration of salt or proteins should be diluted with water before mixing with loading buffer.
- ✓ Do not boil sample after adding loading buffer.