# Protocol

TD-P Revision 2.0

**Creation Date: 2/28/2017**
**Revision Date: 3/6/2019**

## 6X GelRed™ Prestain Loading Buffer Protocol

### Preparation of Prestain Loading Buffer for Electrophoresis

## Introduction

GelRed™ is an ultra-sensitive, extremely stable and environmentally safe fluorescent nucleic acid dye designed to replace the highly toxic ethidium bromide (EtBr). 6X GelRed™ Prestain Loading Buffers are gel loading buffers containing density agents, tracking dyes, and GelRed dye. The 6X prestain loading buffer is added to samples in place of gel loading buffer and eliminates the need to add fluorescent DNA dye to the agarose gel during casting.

Buffers with blue tracking dyes contains two blue electrophoresis tracking dyes that run at approximately 1.5 kb and 200 bp in a 1% agarose gel. Buffers with orange tracking dyes contains an orange electrophoresis tracking dyes that run at approximately 50 bp in a 1% agarose gel.

GelRed™ and EtBr have virtually the same spectra, so you can directly replace EtBr with GelRed™ without changing your existing imaging system. In addition, GelRed™ is far more sensitive than EtBr, which cannot be used in DNA loading buffer to prestain DNA. GelRed™ is compatible with downstream applications such as sequencing and cloning. GelRed™ is efficiently removed from DNA by commercial gel extraction kits or by phenol/chloroform extraction and ethanol precipitation.

A series of safety tests have confirmed that GelRed™ is noncytotoxic, nonmutagenic and nonhazardous at concentrations well above the working concentrations used in gel staining. As a result, GelRed™ can be safely disposed of down the drain or in regular trash, providing convenience and reducing cost in waste disposal.

## Storage/Handling

Store at 4°C. Protect from light. Product is stable for at least 6 months from date of receipt when stored as recommended. The dye can be handled under ambient light during sample preparation and electrophoresis without affecting product performance.

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## Spectral Properties

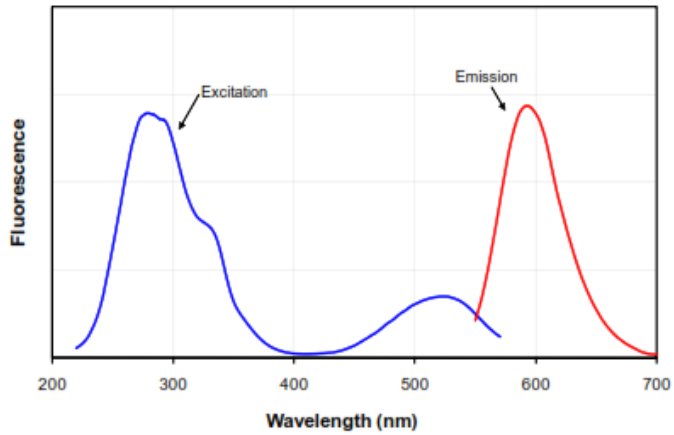


**Figure 1:** Excitation and emission spectra of GelRed™ dye in the presence of dsDNA.

## Materials

- 6X GelRed™ Prestain Loading Buffer with Blue Tracking Dyes (Catalog # G-730)
- 6X GelRed™ Prestain Loading Buffer with Orange Tracking Dye (Catalog # G-735)

## Method

The optimal loading amount of DNA is 50-200 ng DNA per lane. For samples of unknown DNA concentration, we recommend loading ½ or less the volume you would normally run on an ethidium bromide precast gel. Loading more than the recommended amount of DNA may result in smearing or smiling of bands or shifted band migration. If you need to run more than the recommended amount of DNA per lane, or if highly accurate sizing of DNA fragments is required, we recommend using GelRed™ Nucleic Acid Gel Stain, 10,000X in Water (Catalog # G-725) to stain gels after electrophoresis using the post-staining procedure.

1. Prepare agarose gel according to your standard protocol. Do not add ethidium bromide, GelRed™, or any other fluorescent DNA dye to the agarose or buffer.

2. Briefly vortex 6X Gel Red™ Pertain Loading Buffer. Add 6X buffer to DNA samples at a volume ratio of 1:5 (for example, mix 10 µl sample + 2 µl 6X loading buffer). For best results, the 10 µl sample should either contain between 50-100 ng DNA for ladders or between 10-20 ng DNA for samples.

3. Load samples and run gels according to your standard protocol.

4. Visualize bands using a UV transilluminator or other gel documentation system. Gels can be imaged using an ethidium bromide emission filter. SYBR® Green or GelStar™ filters also can be used for gel imaging with equally good results.