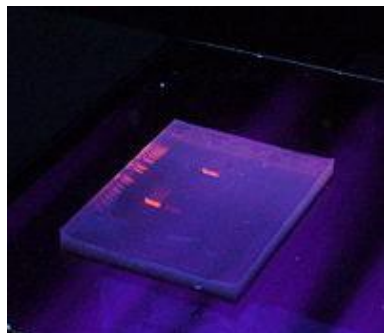


## Safer Alternatives to Ethidium Bromide



Different types of dyes are used to stain nucleic acids in the preparation and use of electrophoresis gels. The hazard properties of various products, and hence the disposal requirements, are very different. While some products are completely safe or less toxic, others are mutagenic and require special handling and disposal procedures. As new products become available it is important to clarify the hazard properties and disposal requirements of these dyes.

[Photo from: [http://en.wikipedia.org/wiki/Ethidium\\_bromide](http://en.wikipedia.org/wiki/Ethidium_bromide)]

### Non-Mutagenic Dyes

SYBR®Safe, GelRed™, GelGreen™, and EvaGreen®. Independent licensed testing laboratories have determined in Ames tests that these dyes are non-mutagenic.

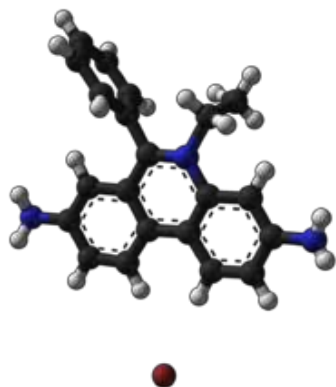
### Mutagenic Dyes

The following dyes have been determined to have mutagenic and/or toxic properties: Ethidium Bromide, Methylene Blue, Crystal Violet, Propidium Iodide, Acridine Orange, SYBR®Green I, SYBR®Green II, SYBR®Gold and GelStar™.

All gels containing these dyes, unwanted dye stock solutions, and all contaminated debris must be handled and disposed as hazardous waste. For details refer to the [Laboratory Pollution Prevention and Hazardous Waste Management Manual](#).

**Learn More (below is a comparison of some commonly used DNA stains):**

### Ethidium Bromide



Ethidium bromide (3,8-Diamino-5-ethyl-6-phenylphenanthridinium bromide) is the classic DNA stain. Ethidium bromide (EtBr) is a flat molecule that fits between adjacent base pairs (intercalates) in the DNA double helix. It has UV absorbance maxima at 300 and 360nm, and can also absorb energy from nucleotides excited at 260nm. The absorbed energy is emitted as orange/yellow light at 590nm. The fluorescence of EtBr is significantly higher when intercalated than it is in aqueous solution.

[Photo from: [http://en.wikipedia.org/wiki/Ethidium\\_bromide](http://en.wikipedia.org/wiki/Ethidium_bromide)]



**Toxicity:** Toxin, mutagen, teratogen and carcinogen according to a variety of tests but effects on higher organisms have not been proven. [Read the MSDS here.](#)

**Protocol:** Can be used in the gel at or as a post-stain at a concentration of 0.5 mg/L.

**Detection:** UV light

**Sensitivity:** Can detect bands of 1-5ng

**Reference:**

<https://www.nationaldiagnostics.com/electrophoresis/article/ethidium-bromide-staining>

### **SYBR® Safe**

SYBR®Safe is a commercial DNA stain manufactured by Invitrogen. It is marketed as being less harmful than ethidium bromide, but this is debatable. Its major advantage is that it is as sensitive as ethidium bromide but does not require UV light for visualization.

**Toxicity:** Documented as less mutagenic than ethidium bromide, but its acute toxicity is higher. You can read the Molecular Probes [white paper on SYBR®Safe toxicity here.](#)

**Protocol:** SYBR®Safe is used as an in-gel stain only. It is supplied in ready-made buffers, 10,000X concentrate in DMSO.

**Detection:** For visualizing fragments required for downstream applications, the best (although more expensive) option is to use a blue light box as the wavelengths used do not cause DNA damage. UV-transilluminators can also be used, although specific filters may be required.

**Sensitivity:** As sensitive as ethidium bromide – bands of 1-5ng should be detectable.

### **GelRed™**

GelRed™ is a commercial DNA stain manufactured by Biotium. It is marketed as being the most safe, sensitive and robust nucleic acid gel stain- less mutagenic than ethidium bromide, but more stable in storage than SYBR®Safe. Like ethidium bromide, GelRed™ is visualized using UV light.

**Toxicity:** Less mutagenic than ethidium bromide. [Read the safety report.](http://www.biotium.com/product/product_info/Safety_Report/GR%20&%20GG%20safety.pdf) ([http://www.biotium.com/product/product\\_info/Safety\\_Report/GR%20&%20GG%20safety.pdf](http://www.biotium.com/product/product_info/Safety_Report/GR%20&%20GG%20safety.pdf))

**Protocol:** Gel red can be used as post stain or in-gel stain. It is supplied in ready-made buffers, GelRed™ 10,000X in H<sub>2</sub>O or DMSO.

**Detection:** UV: excitation at 300nm, emission at 595nm – so conventional UV transilluminators are sufficient.

**Sensitivity:** Bands of 0.25ng can be detected, some researchers have reported from calculations using data available in the [product brochure.](#)

### **Methylene Blue**

Methylene blue is member of the thiazin family of dyes that bind ionically to DNA and RNA. Since its interaction with DNA/RNA is weak, methylene blue is not a very sensitive stain, but has the advantage that is detectable in the visible range. Destaining in water may be required for maximum sensitivity.

**Toxicity:** Possible mutagenicity and reproductive toxicity. Toxic if ingested. [Read MSDS.](#)



**Protocol:** Post strain only, in 0.025% (w/v) methylene blue in water.

**Detection:** Visible light.

**Sensitivity:** 40-100ng bands are reported to be detectable after de-staining. Some researchers reported that only bands of 500ng and over are reliably detectable.

### Crystal Violet

Crystal violet intercalates into DNA in a similar manner to ethidium bromide but is apparently less mutagenic. Its major advantage is that it is detectable in the visible range – so no need for UV exposure.

**Toxicity:** Carcinogen, Mutagen (less so than ethidium bromide). Very toxic by ingestion. [Read MSDS](#).

**Protocol:** Use in gels at a concentration of around 1.2 mg/mL

**Detection:** Visible light

**Sensitivity:** 100-200ng bands are reported to be detectable, but less sensitive than EtBR; some researchers have reported that 2ug+ may be required for clearly visible bands.

### Additional References:

- Propidium Iodide [MSDS](#):
- Acridine Orange [MSDS](#):
- SYBR® Green I [product sheet](#)
- SYBR® Green II [product sheet](#)
- SYBR® Gold [product sheet](#)
- GelStar® [Product Protocol](#) and [MSDS](#):
- EvaGreen® [product information](#) and [Safety Report](#)