

DNA analysis - Electrophoresis

Nucleic acids staining

RNA markers

Interchim offers three RNA molecular weight markers for the calibration of purification and transcription products from such techniques as Northern hybridization, RT-PCR, and cDNA library construction.

RNA Marker, 16S + 23S resolves 2 fragments of ribosomal RNA purified from E. coli. The fragment sizes are approximately 1 776 bp and 3 566 bp.

RNA Marker, 18S + 28S resolves 2 fragments of ribosomal RNA purified from Calf Liver. The fragment sizes are approximately 2 000 bp and 5 300 bp.

Transteps™ RNA Marker is a unique ladder comprised of 10 transcription products, varying in size from 0.5 kb to 9.0 kb.

Description	Cat.#	Qty
RNA Marker, 16S + 23S (2 bands : 1 776 and 3 566 bp)	951560	2.5 mg
RNA Marker, 18S + 28S (2 bands : 2 000 and 5 300 bp)	730410	250 µg
Transteps RNA Marker (10 bands from 0.5 kb to 9.0 kb)	793860	50 µg

GelRed™ Nucleic Acid Gel Stain

Simply the best nucleic acids gel stain!

- ◆ **Superior Sensitivity**
The most sensitive and robust nucleic acid gel stain.
- ◆ **Unsurpassed Thermal Stability, Hydrolytical Stability and Photostability**
Can be microwaved or subjected to other similar heating procedures for making agarose gels ; stable in alkaline or acidic buffers at room temperature; highly photostable.
- ◆ **Improved Safety**
Shown to be much less mutagenic than ethidium bromide by Ames test.
- ◆ **Ultimate Flexibility**
Can be used for either precast or post gel staining; for either agarose gels or polyacrylamide gels; and for either dsDNA or ssDNA or RNA.
- ◆ **Simple Staining Procedure**
Prepare and run precast gels as with EB without having to worry about dye stability; and takes as little as 30 minutes for post staining without the need for destaining.
- ◆ **Minimal Effect on DNA Migration Pattern**
DNA migration pattern in GelRed precast gels similar to that in gels without dyes.
- ◆ **No Need for Filter Change**
Works perfectly well with either a standard EB filter or a "Green" filter.
- ◆ **Perfect Compatibility with a Standard 300 nm UV Transilluminator**
Maximally excited at around 300 nm UV(See Figure 3 for spectra)

Technical tip

Inconsistent results in precast staining?

Question: am I missing something by using Ethidium Bromide (EB)?

Answer: yes. EB has low sensitivity toward lower molecular weight DNA while showing high background in the higher molecular weight DNA region.

Question: why do I sometimes get irreproducible results with other Green DNA stains (Green competitor GelStar™) ?

Answer: both dyes are unstable in the common electrophoresis buffers or in the precast gel matrix.

>>> **The right solution:** Use RedGel™ staining!
RedGel™ has ultimate performance and flexibility solving each of your concerns with conventional stains..

GelRed™ is a superior red fluorescent nucleic acid dye specifically designed for both precast and post gel staining. It has a combination of desirable properties that no other commercial nucleic acid gel stains possess: high sensitivity, extraordinary stability, low toxicity and versatility, accommodating standard to most demanding applications.

Most of the current commercial gel stains are lacking in one or more aspects. For example, although ethidium bromide (EB), the most widely used nucleic acid gel stain, offers acceptable sensitivity in most of the cases, it is a highly mutagenic chemical and its use requires a destaining step to reduce background fluorescence. Green competitor and Gold competitor have been promoted as the most sensitive gel stains by their manufacturer. However, both later have also drawbacks as seen from the feature table below :

Feature	BET	Green I competitor	Gold competitor	GelRed™	
Sensitivity	Good	Good	Excellent	Excellent	[e]
Safety	Highly mutagenic	Non documented	Non documented	Safe (documented)	[d]
Stability	Stable	Degrade	Degrade fairly rapidly	Stable	[a]
Applications				Maximal flexibility	[b]
nucleic acid types	dsDNA, ssDNA, RNA not sensitive for low MW	Only for dsDNA	dsDNA, ssDNA, RNA	dsDNA, ssDNA, RNA	
gel and staining type	Limitations for demanding applications (i.e. precast)	Limitations for demanding applications (i.e. precast)	Not recommended for precast gel staining	* for either precast or post gel staining * for either agarose gels or polyacrylamide gels	
Filter	BET filter	"Green" Filter	"Green" filter	BET or "Green" filter	[c]

[a]: As with EB, precast gels prepared from GelRed™ are stable for long-term storage, whereas precast gels made from Green competitor (or GelStar) degrade rapidly within a day. Especially Gold competitor degrade fairly rapidly under the slightly alkaline condition of the commonly used electrophoresis buffer or in the matrix of precast gels, resulting in unreliable gel staining.

GelRed™ also displays (see Figure 2) consistently superior sensitivity for post gel staining, regardless of the filter used (A vs. C) and storage and handling condition. Gold competitor showed comparable performance only when used fresh from the manufacturer and with a "Green" filter (B vs. D). Following a few freeze-thaw cycles, Gold competitor 10 000X solution degraded significantly, resulting in poor staining (E). Gold competitor 1X solution also degrades (see Figure 4).

[b]: GelRed™ has maximal flexibility, working for either dsDNA or ssDNA or RNA for either precast or post gel staining and for either agarose gels or polyacrylamide gels.

[b1]When used as a precast gel stain, GelRed™ can be microwaved with agarose or be subjected to other heating procedures commonly used in preparing EB precast gels. Unlike EB precast gels, however, GelRed™ precast gels have virtually no background fluorescence and are highly sensitive in detecting low MW DNA fragments. As with EB, precast gels made from GelRed™ can be safely and conveniently stored for later use without compromising the performance of the gels, an utility competitor dyes do not possess (see note [a]).

[b2]When used as a post gel stain, GelRed™ completes the staining in as little as 30 minutes without the need for an extra destaining step. Moreover, since GelRed is hydrolytically stable under either acidic or alkaline condition, its 1X staining solution can be prepared in bulk for later use.

[c]: GelRed™ can be optimally excited with a common 300 nm UV transilluminator. It accommodates either a standard EB filter, or a "Green" filter giving slightly higher signals (see figure 2), as its major excitation peak is around 300 nm and a red emission at around 595 nm (See Figure 3).

[d]: Ames test was performed on both EB and GelRed™ at seven doses (0.6, 1.2, 3, 6, 12, 30 and 60 nmoles) to compare frameshift mutagenicity using S.typhimurium TA98. GelRed™ causes no significant increase of revertant colony number in the absence of metabolic activation in all seven doses tested, indicating lack of mutagenicity without metabolic activation. In the presence of metabolic activation, GelRed™ showed a weak mutagenicity (greatly reduced in comparison to EB).

[e]: GelRed™ is significantly more sensitive than ethidium bromide (EB) for detecting low-level DNA, especially in the lower MW area. see figure 1. For each stain, sensitivity varies depending on nucleic acid type.

We offer GelRed™ as a 10 000X concentrated solution in DMF for your flexibility and also for your convenience GelRed™ 3X solution that can be directly used for post gel staining. We will also soon offer ready-made GelRed™ precast gels for the ultimate convenience.

Description	Cat.#	Qty
GelRed™ nucleic acid gel stain, 10000x in DMF	BQ0410	500 µl
GelRed™ nucleic acid gel stain, 3x solution	BQ0420	4 L

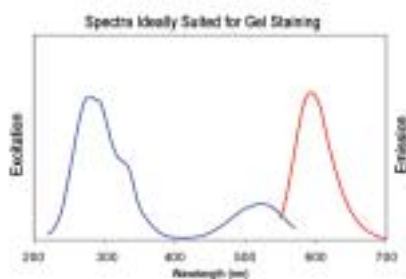


Figure 3: Excitation and emission of GelRed™ in the presence of DNA in PBS buffer.

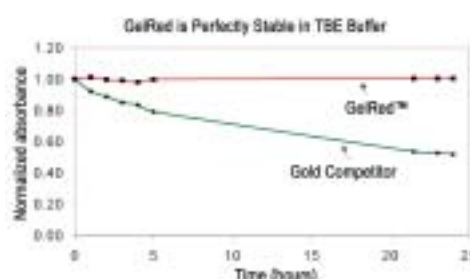


Figure 4: Normalized absorbances of GelRed™ and Gold (Gold competitor) 1 X TBE gel-staining solutions at 500 and 488nm respectively over time at room temperature. The starting absorbance values for GelRed™ and Gold competitor were 0.029 and 0.051, respectively.

Technical tip

The Most Sensitive and Stable
Precast Gel Stain

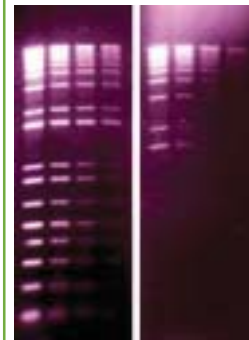


Figure 1. 200 ng, 100 ng, 50 ng and 25 ng (from left to right) of 1 Kb Plus DNA Ladder were electrophoresed on 1% agarose gels precasted with GelRed or EB in 1x TBE. Gels were imaged using 300nm transillumination and photographed with an EB filter and Polaroid 667 black-and-white print films.

The most Sensitive and stable Postcast
Gel stain

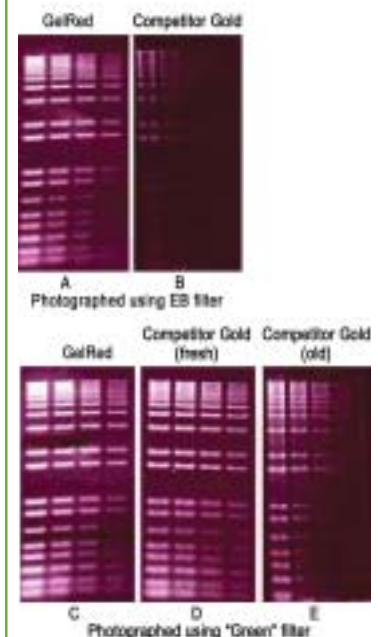


Figure 2. 200 ng, 100 ng, 50 ng and 25 ng (from left to right) of 1 kb Plus DNA Ladder were electrophoresed on 1% agarose gels in 1x TBE and post-stained with GelRed™ (#BQ0410) and Gold competitor, respectively. Gels were imaged using 300nm transillumination and photographed with the indicated filters and Polaroid black-and-white print films. The gel E was stained with a Gold competitor 10 000X solution treated by few freeze-thaw cycles.

DNA analysis - Electrophoresis

Nucleic acids staining

See other DNA probes page D.86 section "DNA stains"

Ethidium bromide

Ethidium bromide is an intercalating dye, which means it inserts itself between the bases that are stacked in the center of the DNA helix. One ethidium bromide molecule binds to one base. As each dye molecule binds to the bases the helix is unwound to accommodate the strain from the dye.

Closed circular DNA is constrained and cannot withstand as much twisting strain as can linear DNA, so circular DNA cannot bind as much dye as can linear DNA.

As Ethidium Bromide is a frame shift mutagen, we provide Ethidium Bromide as a solution that is safer than powder and most convenient to use.

Ethidium Bromide is typically used to stain DNA and RNA in electrophoresis gels. It can also be used to quantitate DNA in solution (250 ng-20 µg/ml), and in conjunction with acridine orange to differentiate between viable, apoptotic and necrotic cells.

Description	Cat.#	Qty
Ethidium Bromide, Biotechnology Grade Powder	06022C	5 g
	06022D	25 g
Ethidium Bromide solution (10 mg/ml solution)	UP89244B	10 ml
Ethidium Bromide, Dropper Bottle (0.625 mg/ml) (A safe solution for easy gel preparation. Use 1 drop per 50 ml of gel solution).	327900	5 ml

Destaining bags

Destaining bags efficiently remove Ethidium Bromide, Coomassie Blue, and other biological dyes from solution. The special adsorbent mixture retains dye molecules in a convenient bag for safe removal and incineration. Each bag will extract up to 5 mg of Ethidium Bromide from solution during an overnight treatment.

Description	Cat.#	Qty
Destaining bags	988422	25 u

SilverSure nucleic acid stain kit

- ◆ Get results in less than 30 minutes.
- ◆ Sensitivity equal to that of ethidium bromide
- ◆ Compatible with most types of agarose
- ◆ Readily visible bands ; no UV required
- ◆ Will not interfere with PCR or cloning DNA from excised bands
- ◆ Safer to use than ethidium bromide; less toxic

SilverSure nucleic acid stain reagents can be used for detecting either DNA or RNA in any type of agarose separation. It is not mutagen, and results are achieved quickly without UV transilluminators.

Description	Cat.#	Qty
SilverSure nucleic acid stain kit	BI9990	1Kit
BromoCresol Green CAS : 76-60-8 ; MW : 698.04 A tracking dye for RNA gels. Also a pH indicator (Yellow to Blue ; range 3.8-5.4)	039820	25g
Bromophenol Blue CAS : 115-39-9 ; MW: 699.99 The standard loading dye to visualize sample for deposition on gels.	039850	25g
	039851	50g
	039852	100g
BromoThymol Blue CAS:76-59-5; MW: 624.4 A pH indicator (Yellow to Blue; range 6.0-7.6)	N12560	25g
	N13191	50g
Phenol Red CAS: 143-74-8 ; MW: 354.38 A pH indicator (Yellow to Red ; range 6.8-8.2)	N13190	25g
	N13191	50g
Xylene Cyanol FF CAS: 2650-17-1; MW: 538.62 A tracking dye for DNA gels	160214	20g

Kit includes :
SilverSure solution A, 5 x 500 ml
SilverSure solution B, 5 x 500 ml
Contains sufficient reagents to stain 25-50 mini-gels.

See also DNA/RNA probes in chapter E : Cell Biology

Loading dyes are designed to optimize loading DNA, RNA and proteins in acrylamide and agarose gels. Loading dyes serve three functions in electrophoresis. First, dyes impart color to the sample to facilitate the loading process. Second, loading dyes increase the sample density to ensure efficient sample distribution into each well. Third, the dyes themselves migrate independently from the samples, allowing the user to estimate the migration of nucleic acids or proteins.

Loading dyes for nucleic acids

Description	Cat.#	Qty
6X Agarose Gel Loading Dye - Ideal for DNA and RNA gels Contains 15% Ficoll in a Tris buffer	741490	5 ml
5X Glycerol Gel Loading Dye - Suited for most DNA and RNA applications	588760 588761	1 ml 5 ml
5X Sucrose Gel Loading Dye - For custom sucrose based dyes Contains 40% Sucrose	662600 662601	1 ml 5 ml
53X Sequencing Gel Loading Dye - For denaturing DNA samples for sequencing Contains Formamide	671860	1 ml

Special dye blend

Description	Cat.#	Qty
25X Loading Dye Base For custom dye preparation	412482	5 ml

Each loading dyes contain :

- ◆ Dye #1 - A light blue dye that migrates at 4 000 base pairs in 1% agarose
- ◆ Dye #2 - An indigo dye that migrates at about 600 base pairs in 1% agarose.
- ◆ Dye #3 - A magenta dye that migrates at 150 base pairs in 1% agarose.

Related product :

Interchim offers a RNA Gel Loading Kit for sample preparation prior to electrophoresis. This comprehensive kit provides all necessary reagents for denaturing and loading RNA samples onto formaldehyde gels buffered with MOPS. These reagents are free from nuclease contamination, and Ultra Pure deionized formamide and DEPC water is used to ensure that the best results can be attained for your experiment. When mixing the sample, the user needs only to dissolve the RNA sample in 10 µl of DEPC water, mixed with 35 µl of denaturing solution and then heated to 65°C for 5 minutes. After the solution has cooled, 5 µl of loading dye is added to the solution. The sample is now ready for loading and electrophoresis.

Description	Cat.#	Qty
RNA gel loading kit	420081	1 Kit