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GelStar™ Nucleic Acid Gel Stain

Instructions for Use

Introduction

GelStar™ Stain is a highly sensitive fluorescent stain for detecting nucleic acids in agarose and polyacrylamide gels. This single stain gives high sensitivity detection of double-stranded or single-stranded DNA and RNA. Gels can be post-stained or alternatively the stain can be added to agarose gels during gel casting. A comparison of GelStar™ Stain with ethidium bromide is shown below. These results were obtained using a 300 nm UV light source and the appropriate filter for each stain.

Nucleic Acid	Sensitivity Increase Compared to Ethidium Bromide
dsDNA	4-16 fold
ssDNA or SSCP DNA	20-80 fold
Glyoxalated RNA	16 fold
Native RNA	3-10 fold
Formaldehyde-denatured RNA	2-3 fold

Storage and Handling

- GelStar™ Stain is supplied as a 10,000X concentrated stock solution in DMSO. Store desiccated at -20°C protected from light. When stored per directions the stock solution is stable for 12 months from the date of receipt.
- Allow time for the stock solution to thaw totally. Removal of stain from partially thawed solutions will result in depletion of stain over time.
- Prepare and store the stain in polypropylene containers such as Rubbermaid® Containers or pipette-tip box lids. The stain may adsorb to glass surfaces and some plastic surfaces, particularly if the surfaces carry residues of anionic detergents or reagents.
- GelStar™ Stain may be diluted in most common electrophoresis buffers with a pH range from 7.0-8.5 or in TE buffer. Staining solutions prepared in water or in buffer with a pH below 7.0 or above 8.5 are less stable and show reduced staining efficiency.
- Diluted stock solutions are stable for several days when stored at 4°C in a polypropylene container protected from light. The number of reuses depends upon the number and size of gels stained and the amount of nucleic acid in the gels.

Cautions

- The DMSO stock solution should be handled with caution as DMSO is known to facilitate entry of organic molecules into tissues. We strongly recommend using double gloves when handling the DMSO stock solutions. Please refer to the GelStar™ Stain MSDS for more details. No data are available on the mutagenicity or toxicity of the GelStar™ Stain.
- **This stain should be treated as a potential mutagen and used with appropriate care. Stain solutions should be disposed of by passing through activated charcoal followed by incineration of the charcoal. Solutions may also be treated using commercially available units designed to bind ethidium bromide.**

Application Notes

- The powder used on some laboratory gloves may contribute to background fluorescence. We recommend the use of powder-free gloves and rinsing gloves prior to handling gels.
- Fibers shed from clothing or lab coats may be fluorescent; be cautious when handling gels.
- Staining of nucleic acids with GelStar™ Stain has minimal impact on blotting efficiency. To ensure efficient hybridization, use of prehybridization and hybridization solutions containing 0.1%-0.3% SDS is important to remove stain retained during transfer.
- GelStar™ Stain can be removed from nucleic acids by ethanol precipitation. Isopropyl alcohol precipitation is less effective at removing the dye; butyl alcohol extraction, chloroform extraction and phenol do not remove the dye efficiently.

Before You Begin

Use a polypropylene container for diluting the stain and staining the gel

Use a 302 or 312 nm UV transilluminator or a blue-light transilluminator such as the Clare Chemical Dark Reader® Transilluminator for dye excitation

Use a GelStar™ Filter for photography

Detection of DNA or RNA in Agarose Gels by Pre-staining

1. Remove GelStar™ Stain from -20°C storage and thaw at room temperature for 10 to 20 minutes.
2. Spin the stain vial briefly in a microcentrifuge to deposit the solution in the bottom of the vial.
3. Prepare the gel solution and cool the agarose solution to 55°C-65°C.
4. Add a volume of stock GelStar™ Stain to the tempered gel solution as indicated below:

For DNA:	For RNA:
Use a final concentration of 1X e.g., 5 µl of stain stock added to each 50 ml of gel solution.	Use a final concentration of 2X, e.g., 10 µl of stain stock added to each 50 ml of gel solution.

5. Mix the gel solution by swirling, stirring, or inversion to thoroughly distribute the stain into the gel solution.
6. **Immediately** cast the gel and allow to solidify. Avoid extended light exposure and extended exposure (>10 min.) to a buffer overlay.
7. Run the gel using your standard protocol.
8. Visualize the results by photography, scanning, or image capture as described, on next page. No destaining is normally required, although we recommend a brief rinse with buffer to minimize deposition of stain on work surfaces.

NOTES:

- The effect of GelStar™ Stain on DNA migration is smaller compared to ethidium bromide, i.e., DNA migration is slower compared to gels with no stain added.
- GelStar™ Stain is sensitive to the presence of particulates in the gel buffer and dust/debris on gel trays. For optimal results, filter the buffer used to prepare the gel solution.
- Including GelStar™ Stain in vertical gels is not recommended as the dye may bind to glass or plastic plates.

Detection of DNA and RNA in Agarose gels by Post-Staining

1. Separate the samples by electrophoresis as normal.
2. Remove GelStar™ Stain from -20°C storage and thaw at room temperature for 10 to 20 minutes.
3. Spin the stain vial briefly in a microcentrifuge to deposit the solution in the bottom of the vial.
4. Dilute stock GelStar™ Stain in buffer in a polypropylene container.

For DNA:	For RNA:
Dilute in TE, TAE, or TBE to give a final concentration of 1X, e.g., 5 µl of stain stock added to each 50 ml of buffer	Dilute stock GelStar™ Stain in 1X MOPS buffer to give a final concentration of 2X, e.g., 10 µl of stain stock added to each 50 ml of buffer.

5. Prepare enough stain solution to cover the gel during staining.
6. Mix the stain solution to distribute the stain thoroughly into the solution.
7. Place the gel and the stain solution in a polypropylene container and incubate with gentle agitation. Protect from direct exposure to strong light during staining. Staining is normally complete within 30 minutes. Exceptionally thick gels (>4 mm) or high concentration gels may require longer staining times for optimal results.
8. Visualize the results by photography, scanning, or image capture as described, on next page. No destaining is normally required, although we recommend a brief rinse with buffer to minimize deposition of stain on work surfaces.

NOTES:

- GelStar™ Stain is compatible with post-staining of DNA in agarose gels >4 mm thick, such as Lonza Reliant™ Precast Agarose Gels.
- GelStar™ Stain gives excellent detection of RNA that has not been denatured, as well as RNA denatured by a variety of methods, e.g., glyoxal, formamide, or formaldehyde. The increase in detection sensitivity in comparison to ethidium bromide staining varies depending upon the sample and gel preparation methods used (see note below and Table on page 1).
- Detection of glyoxalated RNA with GelStar™ Stain is optimal in gels that are ≤4 mm thick. To see the full sensitivity enhancement of GelStar™ Stain, use gels of this thickness or include the stain in the gel for detection.

Detection of Nucleic Acids on Vertical Gels by Post-Staining

1. Separate the samples by electrophoresis as normal.
2. Remove GelStar™ Stain from -20°C storage and thaw at room temperature for 10 to 20 minutes.
3. Spin the stain vial briefly in a microcentrifuge to deposit the solution in the bottom of the vial.
4. Add stock GelStar™ Stain to buffer (TE, TAE, MOPS, or TBE) to give a final concentration of 1X, e.g., 5 µl of stain stock added to each 50 ml of buffer. Prepare enough stain solution to cover the surface of the gel during staining.
5. Mix the stain solution by swirling, stirring, or inversion to distribute the stain thoroughly into the solution.
6. Open the cassette, and leave the gel in place on one plate.
7. Place the plate, gel side up, in a staining container.
8. Gently pour the stain over the surface of the gel; a disposable pipette may be used to help distribute the stain evenly over the gel surface. Do not submerge the gel and plate in staining solution.
9. Incubate for 30 minutes. No destaining is required, although we recommend a brief rinse with buffer to minimize deposition of stain on work surfaces.
10. Visualize the results by photography, scanning, or image capture as described above. For highest sensitivity the gel should be carefully removed from the plate and placed directly on the transilluminator or scanning stage. Alternatively, if a relatively low fluorescence plate is used, the results may be visualized by placing the gel and plate gel side down on the transilluminator and photographing or by scanning the gel directly on the plate.

NOTES:

- As an alternative to the protocol presented for staining gels on the cassette plate, smaller gels such as minigels may be removed from both plates then stained using the protocol for post-staining agarose gels.
- Treatment of one plate with a “release” agent, such as Gel Slick™ Solution (Lonza Catalog No. 50640), increases the ease of separation the glass plates while keeping the gel in place on the other plate for staining.
- Handling or compression of gels (particularly polyacrylamide-type gels) can lead to regions of high background after staining. If possible gels should not be handled directly; use a spatula (or other tool) and a squirt bottle to slide the gel off the plates and into the stain or onto the light box.
- Addition of 50% glycerol to the staining buffer is recommended when using GelStar™ Stain with MDE™ Gels (Lonza Catalog No. 50620) for heteroduplex or SSCP analyses. This minimizes swelling of the gel during staining, and improves gel handling and staining intensity.

Excitation of GelStar™ Stained Nucleic Acids

Either:
Illuminate the gel with a standard UV transilluminator (302 or 312 nm).
Or:
Illuminate the gel with a blue-light transilluminator such as the Clare Chemical Dark Reader® Transilluminator .
Or:
Excite the GelStar™ Stain with an argon laser scanning system. Systems compatible with the detection of SYBR® Green Stain should also be compatible with GelStar™ Stain.

Visualization by Photography

Photograph the gel with the filter and film in the table below. Polaroid® type 55 positive/negative film can be used for photography of gels having relatively strong signals.

Exposure time varies with the strength of the illumination source and the filter used for photography.

Suggested Exposure Conditions For Different Film Types.

Film	*f*-stop	Filter	Exposure
Type 57 or 667	4.5	GelStar™ Filter	2-5 seconds
Type 55	4.5	GelStar™ Filter	15-45 seconds

Visualization by Image Capture systems
GelStar™ Stain is compatible with most CCD and video imaging systems. Due to variations in the filters for these systems you may need to purchase a new filter. Lonza does not sell filters for this type of camera. Contact your systems manufacturer and using the excitation and emission information listed they can guide you to an appropriate filter. The excitation and emission maxima of GelStar™ Stain are 493 nm and 527 nm (532 nm for RNA) respectively.

Ordering Information

Catalog No.	Description	Quantity
50535	GelStar™ Nucleic Acid Stain	2 x 250 µl
50536	GelStar™ Nucleic Acid Stain Photographic Filter	1 each

Related products

DNA Ladders
MetaPhor™ Agarose
NuSieve™ GTG™ Agarose
NuSieve™ 3:1 Agarose
PAGEr™ Gold TBE Precast Gels
SeaKem® GTG™ Agarose
SeaKem® LE Agarose
SeaPlaque™ GTG™ Agarose
MDE™ Gel Solution

For more information contact Scientific Support at (800) 521-0390 or visit our website at www.lonza.com

GelStar™ Stain is manufactured for Lonza Rockland, Inc.

For Research Use Only.
Not for use in diagnostic procedures.