Revised: December 20, 2021

Product Information

EMBER500™ RNA Prestain Loading Dye

Catalog Number: 41032-250uL, 41032

Unit Size:
41032-250uL: 0.25 mL
41032: 4 x 1 mL

Storage and Handling
Store at -20°C and protect from light. Product is stable for at least 6 months from date of receipt when stored as recommended.

This product contains a potentially hazardous nucleic acid binding dye and other hazardous chemicals. Handle using universal laboratory safety precautions and dispose as hazardous waste. Download the safety data sheet (SDS) from the website product page for more information.

Product Description
EMBER500™ RNA Prestain Loading Dye is a convenient reagent for single-step denaturing, staining, and loading of RNA for gel analysis. The loading dye contains EMBER500™, a bright and versatile fluorescent DNA and RNA binding dye with broad instrument compatibility. EMBER500™ prestaining gives much brighter signal and higher sensitivity than ethidium bromide prestaining (Figure 1). The dye stains RNA and DNA, allowing the detection of contaminating genomic DNA in purified RNA samples (Figure 2). The prestaining procedure is simpler, faster, and more sensitive than post-electrophoresis gel staining, and allows the use of a native agarose gel instead of a complicated and hazardous denaturing gel. EMBER500™ staining can be imaged using a UV transilluminator with standard filters, and also is compatible with blue LED gel imagers, which eliminate UV exposure hazards (Figure 3).

The loading dye is supplied at 2X concentration. It contains formamide for denaturing RNA, and the electrophoresis tracking dyes bromophenol blue and xylene cyanol, which migrate in agarose gels at ~300 bp and ~3 kb respectively.

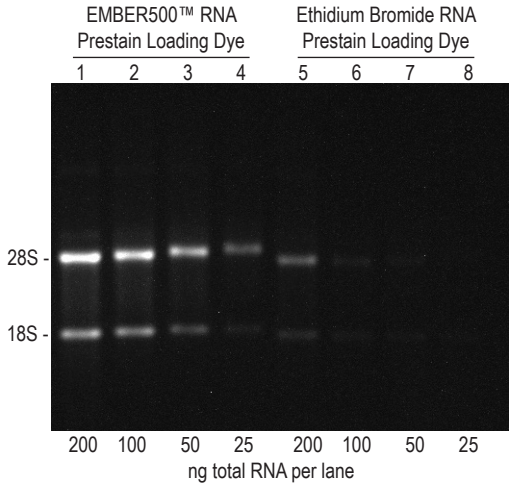

Figure 1. EMBER500™ staining is much more sensitive than ethidium bromide. Human total cellular RNA gel samples were prepared with EMBER500™ RNA Prestain Loading Dye (lanes 1-4) or RNA loading dye with 250 ug/mL ethidium bromide (EtBr) (lanes 5-8), heated at 70°C for 10 minutes, and then separated on a 1% agarose/1X TBE non-denaturing gel. Loading amounts (total ng RNA per lane) are indicated below the gel. The gel was imaged using a UVP GelDoc-iT® imaging system with a UV transilluminator and EtBr filter. The positions of the 28S (5 kb) and 18S (1.9 kb) ribosomal RNA bands are indicated.

Considerations for Use
- When using this product, precautions should be taken to avoid contamination with RNase. Use nuclease-free tubes, tips, and reagents.
- EMBER500™ dye stains both DNA and RNA with high sensitivity, and can be used to evaluate both RNA integrity and contaminating genomic DNA in total RNA samples (Figure 2).
- EMBER500™ RNA Prestain Loading Dye works best with higher molecular weight RNA samples, such as total cellular RNA or RNA fragments larger than 500 bp.
- This loading dye contains formamide to maintain samples in a denatured state after heating. We recommend running samples on a non-denaturing 1% agarose gel in 1X TBE buffer.
- EMBER500™ prestaining is not recommended for analyzing low molecular weight RNA or ssDNA, or for staining denaturing gels such as formaldehyde, glyoxal, or urea gels. For these applications, we recommend performing post-staining with Oxazole Gold (see Related Products).
- We recommend loading 100-200 ng per lane of total RNA, or 200-500 ng per lane of ssRNA ladder. Loading more than the recommended amount may result in migration shift of bands. However, detection of smaller fragments (less than 1000 bp) may require loading higher loading amounts.
- The loading dye is supplied at 2X concentration, to be added in equal volume to samples before loading. However, you may also dilute your RNA directly in the undiluted loading dye at a ratio of up to 1:10 (1 part RNA to 9 parts loading dye) with similar results (Figure 3).
- Nucleic acid dyes like EMBER500™ are compatible with northern blotting, and are retained on RNA after transfer to membranes. Dyes may reduce probe hybridization signal; 0.1-0.3% SDS may be included in the blot equilibration buffer to remove nucleic acid dyes before hybridization.
- EMBER™500 staining can be imaged using a blue LED gel imager or a UV transilluminator. A filter for green fluorescent dyes will give the brightest signal, but the dye also can be imaged using an ethidium bromide (EtBr) filter with good results (Figure 3).

Protocol for Agarose Gel Electrophoresis of RNA

Materials required but not provided
- Agarose, LE (Cat. no. 41028)
- 5X TBE (Cat. no. 41006)
- Water, Ultrapure Molecular Biology Grade (Cat. no. 41024)

Procedure
1. Prepare a 1% agarose gel in 1X TBE buffer.
2. Dilute your RNA samples for electrophoresis in RNase-free water (see recommended loading amounts under Considerations for Use).
3. Add an equal volume of EMBER500™ RNA Prestain Loading Dye to each RNA sample and mix well. For example, add 5 uL prestain loading dye to 5 uL RNA sample.

   Note: Samples can be mixed with up to 10 volumes of prestain loading dye (for example,1 uL of RNA sample with 9 uL of prestain loading dye).
4. Heat samples at 70°C for 5-10 minutes.
5. Load samples on the gel. Perform electrophoresis in 1X TBE buffer. The recommended voltage for electrophoresis of RNA is 5 volts per cm of distance between the electrodes on the gel box.
6. Image fluorescence on a UV transilluminator or a blue LED gel imager. See filter recommendations under Considerations for Use.

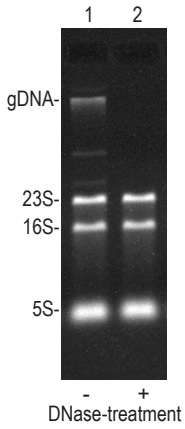

Figure 2. EMBER500™ can be used to evaluate RNA purity. Total RNA was extracted from *E. coli* without DNase-treatment (lane 1) or with DNase-treatment (lane 2) to remove genomic DNA. RNA samples were prestained with EMBER500™ and then separated on a 1% agarose/1X TBE gel at 250 ng per lane. The position of the genomic DNA (gDNA) band and ribosomal RNA bands (23S, 16S, and 5S) are indicated to the left of the gel.

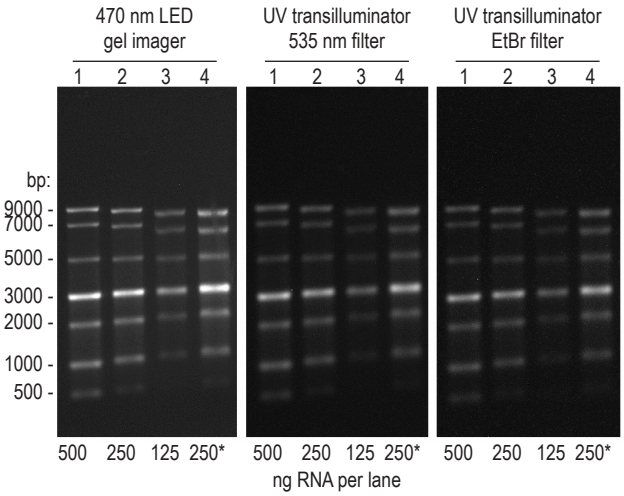

Figure 3. EMBER500™ is compatible with most commonly used gel imagers. A dilution series of NEB ssRNA ladder was prepared using EMBER500™ RNA Prestain Loading Dye, heated at 70°C for 10 minutes, and then separated on a 1% agarose/1X TBE non-denaturing gel. An equal volume of loading dye to RNA sample was used for lanes 1-3. The sample in lanes 4 was mixed with loading dye at a ratio of 1 part RNA solution to 9 parts loading dye. The total amount of RNA loaded per lane (ng) is shown below the gel, and molecular weights for ssRNA ladder bands are shown on the left. The same gel was imaged using a Thermo Fisher Safe Imager™ 2.0 Blue-Light Transilluminator in a UVP GelDoc-iT® darkroom with GelCam 310 2.0 camera (left, 0.5 second exposure time), with a UV transilluminator and 535 nm filter (center, 1 second exposure time), or with a UV transilluminator and EtBr filter (right, 2 second exposure time).

Related Products

Catalog number	Product
41028	Agarose LE, Ultra-Pure Molecular Biology Grade
41006	TBE Buffer, 5X (4L Cubitainer®)
41024-4L	Water, Ultrapure Molecular Biology Grade (4L Cubitainer®)
40094	Oxazole Gold (SYBR® Gold), 10,000X in DMSO
40086	Thiazole Green (SYBR® Green I), 10,000X in DMSO
31073	AccuBlue® Broad Range RNA Quantitation Kit
CD504	RNAstorm™ RNA Isolation Kit
CD501	RNAstorm™ Kit for Isolation of RNA from FFPE Tissue Samples
41011	GelRed® Prestain Plus 6X DNA Loading Dye
41001	GelRed® Nucleic Acid Gel Stain, 3X in Water
41003	GelRed® Nucleic Acid Gel Stain, 10,000X in Water
41029	GelRed® Agarose LE
41005	GelGreen® Nucleic Acid Gel Stain, 10,000X in Water
31022	Ready-to-Use 1 kb DNA Ladder
31032	Ready-to-Use 100 bp DNA Ladder
31041	Forget-Me-Not™ EvaGreen® qPCR Master Mix (2-Color Tracking)
31043	Forget-Me-Not™ Universal Probe qPCR Master Mix
29087	VeriFluor™ Far-Red Passive Reference Dye, 400X in Water
31000	EvaGreen® Dye, 20X in Water
31077	EvaGreen® Plus Dye, 2000X in DMSO
31079	EvaRuby™ Dye, 20X in Water

Please visit our website at www.biotium.com for information on our life science research products, including environmentally friendly EvaGreen® qPCR master mixes, fluorescent CF® Dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.