

# Product Information

## EMBER™ Ultra RNA Gel Kit

**Unit Size:** 10 or 30 gels per kit.

### Kit Contents

Component	41044-T 10 gels*	41044 30 gels*
EMBER™ Ultra Precoated Agarose	99880-5g 1 x 5 g bottle	99880-15g 1 x 15 g bottle
EMBER™ Ultra RNA Loading Dye	99882-1.5mL 1 x 1.5 mL	99882-1.5mL 3 x 1.5 mL

\*The number of gels per kit is based on a 9x11 cm, 1% agarose mini gel (0.5 g agarose in 50 mL buffer per gel). The actual number may vary depending on gel size.

### Storage and Handling

Store EMBER™ Ultra RNA Loading Dye at -20°C. Store EMBER™ Ultra Precoated Agarose at room temperature. Protect both components from light. Product is stable for at least 12 months from date of receipt when stored as recommended. The kit components contain a potentially mutagenic DNA binding dye. Handle using universal laboratory safety precautions and dispose of gels as hazardous waste according to your local regulations.

Shake the bottle of EMBER™ Ultra Precoated Agarose to mix well before use. Warm the EMBER™ Ultra Loading Dye to room temperature and vortex to mix before use.

### Product Description

The EMBER™ Ultra RNA Gel system provides superior sensitivity and resolution for RNA gel electrophoresis in a fast and simple agarose gel format. Along with being much more sensitive than any other RNA gel stains, EMBER™ Ultra also does not require a post-electrophoresis staining step. Simply cast your agarose gel using the EMBER™ Ultra Precoated Agarose, and prepare your samples in the denaturing EMBER™ Ultra RNA Loading Dye. Bands can be imaged immediately after electrophoresis.

EMBER™ Ultra Precoated Agarose is ultra-pure molecular biology grade agarose with low electroendosmosis that is precoated with Biotium's fluorescent EMBER™ Ultra Dye. EMBER™ Ultra Precoated Agarose gives excellent results at percentages between 1% and 5% agarose, for analyzing different sizes of RNA.

EMBER™ Ultra Dye has green fluorescence with excitation/emission at 500/530 nm when bound to nucleic acids. For best results, we recommend imaging with a Biotium's Gel-Bright™ Laser Diode Gel Illuminator or a blue light illuminator. Gels can also be imaged using a UV transilluminator equipped with a SYBR® filter (recommended) or EtBr filter (with lower signal).

The EMBER™ Ultra RNA Loading Dye is a formamide-containing denaturing RNA loading dye specifically formulated to work with EMBER™ Ultra Precoated Agarose to provide high sensitivity fluorescent staining of RNA in a fast and convenient agarose gel. The loading dye contains blue and orange tracking dyes that migrate at ~1500 bp (blue) and ~50 bp (orange) in a 1% agarose gel.

**Note:** DNA will also stain on EMBER™ Ultra RNA Gels, so any contaminating DNA in your RNA sample will also be detected. See our EMBER™ Ultra DNA Agarose Gel Kit for highly sensitive detection of DNA on agarose gels.

### Experimental Protocols

**Important:** For best results, use the EMBER™ Ultra Precoated Agarose in combination with EMBER™ Ultra Loading Dye. Using other loading dyes with EMBER™ Ultra Precoated Agarose, or using EMBER™ Ultra Loading Dye with other gel types, will result in aberrant gel migration.

When using this product, precautions should be taken to avoid contamination with RNase. Use nuclease-free tubes, tips, and reagents. We recommend cleaning your workspace, gel boxes, and pipettes with RNase-X™ (see Related Products) or a similar RNase decontamination solution before working with RNA.

### Materials required but not provided

- 1X TBE Buffer

#### 1. Gel casting

**Note:** We recommend melting the agarose and casting the gel on the day of use.

- 1.1 Shake the closed bottle of EMBER™ Ultra Precoated Agarose to thoroughly mix the powder.
- 1.2 Weigh out the EMBER™ Ultra Precoated Agarose for the desired gel percentage as shown below. The RNA size ranges are to be used as a general guide only, optimal separation may vary depending on the specific type of RNA sample.

RNA fragment size	Gel percentage	Agarose per 50 mL
500 to >9000 bases	1%	0.5 g
50 to 1000 bases	2%	1 g
< 50 bases	5%	2.5 g

- 1.3 Add the agarose to 1X TBE buffer in an Erlenmeyer flask that is large enough to allow the solution to boil (for example, use a 125 mL or larger flask to make 50 mL of molten agarose).

**Note:** Do not add any additional fluorescent dye to the agarose.

- 1.4 Microwave for 1 minute, swirl to mix thoroughly, and microwave for an additional minute.

**Caution!** Handle heated solutions of agarose with care to avoid boiling over and the risk of burns.

- 1.5 Swirl to mix and make sure agarose is completely dissolved.
- 1.6 Allow the solution to cool for ~1 minute.
- 1.7 Pour the gel and insert the comb.
- 1.8 Allow the gel to set and cool completely before removing the comb.

#### 2. Sample preparation and electrophoresis

- 2.1 **Recommended sample loading amounts:** Avoid overloading samples, which may result in distorted bands. Dilute your RNA samples in RNase-free water or TE buffer if necessary.

- For blue light illuminator (Biotium's Gel-Bright™ Laser Diode Gel Illuminator, Thermo's Safe Imager™, or similar): Load 20-100 ng RNA per 1 mm gel lane.
- For use with a UV transilluminator with SYBR® filter: Load 50-200 ng RNA per 1 mm gel lane.
- For samples of unknown concentration: Use 5 uL of sample as a starting point for optimization.

- 2.2 Warm the EMBER™ Ultra RNA Loading Dye to room temperature and vortex to mix thoroughly before use.

- 2.3 Add an equal volume of EMBER™ Ultra RNA Loading Dye to each RNA sample. For example, combine 5 µL of RNA sample with 5 µL of EMBER™ Ultra RNA Loading Dye and mix well.
- Note:** Use EMBER™ Ultra RNA Loading Dye in all of your samples, including RNA ladders. The loading dye may be added to samples already containing another RNA Loading Dye, such as ready-to-load RNA ladders.
- 2.4 Heat the samples at 70°C for 5-10 minutes to denature the RNA.
- 2.5 Briefly centrifuge samples to collect the contents at the bottom of the tubes.
- 2.6 Load the entire sample volume on the gel.
- 2.7 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.
- 2.8 Image the gel on a blue light gel illuminator or UV transilluminator with a SYBR® filter.

### Troubleshooting

Problem	Solutions
Distorted Bands	<ul style="list-style-type: none"> <li>Avoid overloading samples. See loading recommendations under “Sample preparation and electrophoresis.”</li> <li>Ensure samples are heated evenly during the denaturation step. If using a dry bath heat block to heat samples, use tubes that fit snugly in the heat block holes.</li> <li>Only use EMBER™ Ultra Precoated Agarose together with EMBER™ Ultra RNA Loading Dye. Using EMBER™ Ultra components in combination with other types of agarose or loading dyes will result in distorted bands.</li> <li>Use the recommended buffer system and voltage. Lower voltage and 1X TBE buffer are recommended for RNA gels to minimize heat during electrophoresis.</li> </ul>
RNA is degraded (smeared bands)	<ul style="list-style-type: none"> <li>Use nuclease-free tubes, tips, and reagents. We recommend cleaning your workspace, gel boxes, and pipettes with RNase-X™ (see Related Products) or a similar RNase decontamination solution before working with RNA.</li> </ul>
Total RNA contains significant genomic DNA	<ul style="list-style-type: none"> <li>Include a DNase digestion step to remove contaminating genomic DNA.</li> <li>Capacity of RNA purification method might be exceeded. Follow the sample size recommendations for your specific purification system.</li> </ul>

### Related Products

Cat. No.	Product
22028	RNase-X™ Decontamination Solution
E90005	Gel-Bright™ Laser Diode Gel Illuminator
41043	EMBER™ Ultra DNA Gel Kit
41024	Water, Ultrapure Molecular Biology Grade
41006	1X TBE Buffer
31073	AccuBlue® Broad Range RNA Quantitation Kit
CD504	CELLDATA RNAsort™ Fresh Cell and Tissue RNA Isolation Kit
CD506	CELLDATA RNAsort™ 2.0 FFPE RNA Extraction Kit
CD508	CELLDATA DNAsort™ RNAsort™ 2.0 Combination Kit
28001	ExoBrite™ EV Total RNA Isolation Kit
99850	RNA Standard
31065	RNase-Free Calf Thymus DNA, 1 mg/mL
31041	Forget-Me-Not™ EvaGreen® qPCR Master Mix (2-Color Tracking)
31043	Forget-Me-Not™ Universal Probe qPCR Master Mix

Please visit our website at [www.biotium.com](http://www.biotium.com) for information on our products for RNA workflows and applications, including RNA extraction kits for fresh cells and FFPE tissues, RNA quantitation kits, and RNA gel stains.

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