

# TRIzol<sup>®</sup> Max<sup>™</sup> Bacterial RNA Isolation Kit

Catalog Numbers: 16122-012, 16096-020, 16096-040

## Description

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### Types of Products

This manual is supplied with the following products. Twenty milliliters of Max Bacterial Enhancement Reagent is sufficient to perform 100 isolations.

Product	Contents	Cat. no.
Max Bacterial Enhancement Reagent	20 mL	16122-012
TRIzol <sup>®</sup> Max <sup>™</sup> Bacterial RNA Isolation Kit	20 mL Max Bacterial Enhancement Reagent 100 mL TRIzol <sup>®</sup> Reagent	16096-020
TRIzol <sup>®</sup> Max <sup>™</sup> Bacterial RNA Isolation Kit	2 × 20 mL Max Bacterial Enhancement Reagent 200 mL TRIzol <sup>®</sup> Reagent	16096-040

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### Storage

The Max Bacterial Enhancement Reagent and TRIzol<sup>®</sup> Reagent are shipped at room temperature.

Store Max Bacterial Enhancement Reagent at room temperature. **Do not** store at 4°C or -20°C. If a precipitate is formed in the Max Bacterial Enhancement Reagent, heat the reagent at 65°C until the precipitate is dissolved.

Store TRIzol<sup>®</sup> Reagent at room temperature.

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### About the Kit

The Max Bacterial Enhancement Reagent is designed for use with TRIzol<sup>®</sup> Reagent to improve the isolation of intact total RNA up to 3-fold from gram-positive and gram-negative bacteria. The Max Bacterial Enhancement Reagent is a ready-to-use solution composed of chelating agents, detergent, and a buffer, and is used as an efficient pre-treatment buffer for bacterial cells prior to RNA isolation with TRIzol<sup>®</sup> Reagent. The use of Max Bacterial Enhancement Reagent with TRIzol<sup>®</sup> inactivates endogenous RNases and promotes protein denaturation improving the RNA quality and integrity.

Bacterial cells are pre-treated with Max Bacterial Enhancement Reagent and incubated at high temperature. TRIzol<sup>®</sup> Reagent is then added to dissolve the cell components and maintain RNA integrity. Addition of chloroform followed by centrifugation separates the lysate into an aqueous phase containing RNA and an organic phase. RNA is recovered from the aqueous phase by precipitation with isopropanol. The final RNA pellet is dissolved in RNase free/DEPC-treated water.

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### Advantages

Using Max Bacterial Enhancement Reagent with TRIzol<sup>®</sup> Reagent to isolate total RNA from bacteria offers the following advantages:

- Higher yields due to improved lysis of bacterial cells and minimal RNA degradation
  - Improves total RNA isolation from gram-positive and gram-negative bacteria
  - Minimal genomic DNA contamination of the purified RNA sample
  - Eliminates the need for time consuming mechanical and/or enzymatic cell lysis steps
  - Reliable performance of the high-quality purified total RNA in downstream applications
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### Intended Use

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

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## Using Max Bacterial Enhancement Reagent



When working with TRIzol® Reagent, use a laboratory coat, safety glasses, and gloves. Avoid contact with skin or clothing. Use in a chemical fume hood and avoid breathing vapor.

### Materials Needed

- TRIzol® Reagent (supplied with Cat. nos. 16096-020 and 16096-040)
- Bacterial cells
- Chloroform, chilled
- Isopropanol, chilled
- 75% Ethanol
- RNase-free water (page 3)
- Heating block set at 95°C
- Microcentrifuge and sterile microcentrifuge tubes

### General Handling of RNA

Observe the following guidelines to prevent RNase contamination:

- Use disposable, individually wrapped, sterile plasticware.
- Use only sterile, new pipette tips and microcentrifuge tubes.
- Wear latex gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin.
- Always use proper microbiological aseptic techniques when working with RNA.
- Use RNase AWAY® Reagent (page 3) to remove RNase contamination from surfaces.

### Procedure

1. Inoculate bacteria into a suitable medium (add appropriate antibiotic, if needed).
2. Incubate the culture with shaking at the appropriate temperature for the desired time to obtain log phase cells.
3. Transfer 1.5 mL of bacterial culture (up to  $1 \times 10^8$  cells) to a pre-chilled microcentrifuge tube.
4. Centrifuge the tube at  $6,000 \times g$  for 5 minutes at 4°C in a microcentrifuge.
5. During centrifugation, preheat 200 µL Max Bacterial Enhancement Reagent to 95°C.
6. After centrifugation, decant the supernatant and resuspend the cell pellet in preheated 200 µL Max Bacterial Enhancement Reagent from the previous step. Mix well by pipetting up and down.
7. Incubate the tube at 95°C for 4 minutes.
8. Add 1 mL TRIzol® Reagent to the lysate and mix well.
9. Incubate the tube at room temperature for 5 minutes. Proceed to **Phase Separation**, below.

### Phase Separation

1. Add 0.2 mL cold chloroform and mix by shaking the tube vigorously by hand for 15 seconds.
2. Incubate the tube at room temperature for 2–3 minutes.
3. Centrifuge the samples at  $12,000 \times g$  for 15 minutes at 4°C.

After centrifugation, the mixture separates into a lower red, phenol-chloroform phase, an interphase, and a colorless aqueous phase containing RNA. The volume of the aqueous phase is ~400 µL. Proceed to **Precipitating RNA**, next page.

**Note:** Isolation of DNA and proteins from the interphase and phenol phase after RNA isolation has not been tested and is not recommended with the Max Bacterial Enhancement Reagent.

## Using Max Bacterial Enhancement Reagent

### Precipitating RNA

1. Transfer ~400  $\mu$ L of the colorless, upper phase containing RNA to a fresh tube.
2. Add 0.5 mL cold isopropanol to the aqueous phase to precipitate RNA. Mix by inverting the tube.
3. Incubate the tube at room temperature for 10 minutes.
4. Centrifuge at  $15,000 \times g$  for 10 minutes at  $4^{\circ}\text{C}$ .
5. Remove the supernatant carefully without disturbing the RNA pellet (a gel-like pellet formed at the side and bottom of the tube).
6. Resuspend the pellet in 1 mL 75% ethanol. Mix well by vortexing.
7. Centrifuge at  $7,500 \times g$  for 5 minutes at  $4^{\circ}\text{C}$ . Discard the supernatant.
8. Air-dry the RNA pellet. Do not dry the RNA pellet by centrifugation under vacuum.
9. Resuspend the RNA pellet in 50  $\mu$ L RNase-free water by pipetting up and down, and incubating for 10 minutes at  $60^{\circ}\text{C}$ , if needed.

### Estimating RNA Quantity

Determine the purified total RNA quantity as described below.

1. Dilute an aliquot of the total RNA sample in 10 mM Tris-HCl, pH 7.0. Mix well. Transfer to a 1-cm path length cuvette.
2. Determine the absorbance of the solution at 260 nm using a spectrophotometer blanked against 10 mM Tris-HCl, pH 7.0.
3. Calculate the amount of total RNA using the following formula:  

$$\text{Total RNA } (\mu\text{g}) = A_{260} \times 40 \mu\text{g} / (1 A_{260} \times 1 \text{ mL}) \times \text{dilution factor} \times \text{total sample volume (mL)}$$

### Expected Yield

The yield of total RNA isolated from 1.5 mL bacterial cells ( $\sim 1 \times 10^8$  cells) using the Max Bacterial Enhancement Reagent with TRIzol<sup>®</sup> Reagent is  $>20 \mu\text{g}$  for *E. coli* (gram-negative bacteria) and  $\sim 3 \mu\text{g}$  for *Lactococcus lactis* (gram-positive bacteria).

Agarose gel electrophoresis of the purified RNA shows distinct 16S and 23S ribosomal bands.

### Troubleshooting

Review the information below to troubleshoot your experiments with the Max Bacterial Enhancement Reagent.

Problem	Cause	Solution
Low RNA yield	Incomplete lysis	Incubate the sample at $95^{\circ}\text{C}$ for 5 minutes after adding Max Reagent to facilitate cell lysis.
	Incorrect phase transferred	The RNA is in the colorless, aqueous phase. Use this phase for precipitating RNA.
	Incomplete dissolution of the final RNA pellet	Be sure to completely dissolve the final RNA pellet. If needed, heat at $60^{\circ}\text{C}$ for 10 minutes.

### Accessory Products

The following products are available from Invitrogen. For more details on these products, visit [www.invitrogen.com](http://www.invitrogen.com) or contact Technical Support.

Product	Quantity	Cat. no.
TRIzol <sup>®</sup> Reagent	100 mL	15596-026
	200 mL	15596-018
RNase AWAY <sup>®</sup> Reagent	250 mL	10328-011
UltraPure <sup>™</sup> DNase/RNase-Free Distilled Water	500 mL	10977-015
UltraPure <sup>™</sup> DEPC-treated Water	1 L	750023



## Product Qualification and Purchaser Notification

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### Product Qualification and SDS

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to [www.invitrogen.com/support](http://www.invitrogen.com/support) and search for the Certificate of Analysis by product lot number, which is printed on the box.

Safety Data Sheets (SDSs) are available at [www.invitrogen.com/sds](http://www.invitrogen.com/sds).

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