

Bacterial Enhancement Reagent

Catalog Numbers

Bacterial Enhancement Reagent 20ml BIO-38037

Exp. Date: See vial

Batch No.: See vial

Storage and Stability:

The Bacterial Enhancement Reagent should be stored at room temperature. Do not store at +4°C or -20°C. If a precipitate is formed, heat at 65°C until the precipitate is dissolved.

Shipping:

Bacterial Enhancement Reagent at ambient temperature

Safety precautions:

Harmful if swallowed. Irritating to eyes, respiratory system and skin. Please refer to the material safety data sheet for further information.



Store at Room Temperature

Notes:

1. This product insert is a declaration of analysis at the time of manufacture.
2. Research Use Only.

Features

- Improved lysis of bacterial cells for RNA isolation
- Quick isolation of high-quality RNA
- No mechanical or enzymatic lysis steps required

Applications

- RNA isolation from Gram-positive and Gram-negative bacteria

Description

The Bacterial Enhancement Reagent is a ready-to-use solution used to pre-treat Gram-positive and Gram-negative bacterial cells prior to isolation of high-quality RNA using TRIsure™. The combination of the Bacterial Enhancement Reagent and TRIsure improves the quality and integrity of the RNA by inactivating endogenous RNases and promoting protein degradation.

The bacterial cells are initially pre-treated with the Bacterial Enhancement Reagent and incubated at high temperature. TRIsure reagent is then added to dissolve the cell components and maintain the integrity of the extracted RNA.

The isolated RNA is suitable for any downstream application such as RT-PCR, hybridization assays, or *in vitro* translation.

Protocol for the Isolation of RNA using Bacterial Enhancement Reagent and TRIsure

Reagents Required:

- TRIsure
- Bacterial Enhancement Reagent
- Chloroform
- Isopropyl alcohol (chilled)
- 75% Ethanol
- RNase free Water

1. Homogenization

For most bacteria grow log phase culture, for yeast cells or for bacteria with long generation times grow over night culture. Transfer up to 1.5ml bacterial culture (at log phase up to 1×10^8 cells) to a pre-chilled microcentrifuge tube. Centrifuge samples at 6,000 x g for 5 minutes at 2-8°C in a microcentrifuge. During centrifugation pre-heat 200µl Bacterial Enhancement Reagent to 95°C.

After centrifugation discard the supernatant and resuspend the cells in 200µl pre-heated Bacterial Enhancement Reagent. Mix well by pipetting up and down.

Incubate at 95°C for 4 minutes. Add 1ml TRIsure reagent to the lysate and mix well.

2. Phase Separation

Incubate samples for 5 minutes at room temperature. Add 0.2 ml of chloroform per 1 ml of TRIsure used. Cap tubes securely and shake vigorously by hand for 15 seconds.

Incubate samples for 2-3 minutes at room temperature. Centrifuge samples at 12,000 x g for 15 minutes (or 2600 x g for 20-30 minutes) at 2-8°C. The sample will separate into a pale green, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase that contains the RNA.

3. RNA Precipitation

Transfer the aqueous phase very carefully, without disturbing the interphase to another tube. Precipitate the RNA by mixing with cold isopropyl alcohol. Use 0.5 ml of isopropyl alcohol per 1 ml of TRIsure used. Incubate samples for 10 minutes at room temperature then centrifuge at 12,000 x g for 10 minutes (or 2600 x g for 20-30 minutes) at 2-8°C.

Note: For small quantities of cells, RNase-free Glycogen Co-precipitant (BIO-37077) can be added to the aqueous phase before addition of isopropyl alcohol to aid RNA precipitation. Add 5-10µg of Glycogen per 800µl of TRIsure.

4. RNA Wash

Remove the supernatant. Wash the pellet once with 75% ethanol, adding at least 1 ml of ethanol per 1 ml of TRIsure used. Vortex samples and centrifuge at 7500 x g for 5 minutes at 2-8°C.

Note: At this stage, samples can be stored for one week at 2-8°C, or 12 months at -5 to -20°C.

5. Re-dissolving the RNA

Air-dry the pellet and dissolve in RNase free water (BIO-37080) or DEPC-treated Water (BIO-38030) by pipetting the solution up and down. Incubate for 10 minutes at 55-60°C if necessary.

Store RNA between -20°C and -70°C.

Associated Products

Product Name	Pack Size	Catalog No.
TRIsure	100ml	BIO-38032
RiboLadder Short	25 Lanes	BIO-33060
RiboLadder Long	25 Lanes	BIO-33061
BioScript™	10,000 Units	BIO-27036
RNA Loading Buffer	1ml	BIO-38025

Troubleshooting Guide

Problem	Possible Cause	Recommendation
Genomic DNA contamination*	Incomplete lysis or homogenisation	Homogenise cells thoroughly in Bacterial Enhancement Reagent by pipetting up and down. Incubate Homogenate for 5 min at 95°C
	Insufficient volume of TRIsure used	Ensure that 1 ml TRIsure 5 x 10 ⁸ cells is used. If problem persists, increase TRIsure volume by 1.5x
	Contamination of interphase layer during separation of the RNA-containing aqueous layer	Pipette off the aqueous phase very carefully. It is important that none of the white interphase is transferred into your RNA sample, so we recommend that you leave the lower part of the aqueous phase intact
Low RNA yield	Incomplete lysis or homogenisation	Homogenise cells thoroughly in Bacterial Enhancement Reagent by pipetting up and down . Incubate Homogenate for 5 min at 95°C
	Incorrect phase transfer	The RNA is in the upper, colorless phase. Transfer this phase for precipitation of RNA
	Loss of pellet	If starting sample is small, the RNA pellet may not be easily visualised after isopropyl alcohol precipitation, so care must be taken when removing the supernatant from the pellet
	Incomplete solubilisation of final RNA pellet	Ensure RNA pellet is completely dissolved in solution. If necessary incubate for 5-10 min at 60°C
RNA degradation	RNase contamination	The protocol must be carried out carefully in a DNA-free, RNase-free environment. Ensure all pipettes, tips, tubes and work areas are free from RNases and wear gloves. Addition of RNase Inhibitor (BIO-65028) to the extracted RNA sample can help prevent degradation of the sample

* If downstream applications could be affected by small amounts of DNA, we recommend an additional step of treating the RNA sample with RNase free DNase I

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