DNase I Treatment of Purified RNA in Solution for ISOLATE II Biofluids RNA Kit

Bioline

Abstract

The ISOLATE II Biofluids RNA Kit isolates total RNA with minimal amounts of genomic DNA contamination using the supplied Genomic DNA Removal Column. However, additional DNase I treatment may be required in certain cases e.g. the amount of genomic DNA in the sample exceeds the capacity of the Genomic DNA Removal Column, or performing a highly sensitive application.

The on-column DNase I digestion results in minimal residual DNA, undetectable in most downstream applications. For the most sensitive applications, DNA digestion in solution is recommended to eliminate even traces of contaminating DNA. Stringent RNase control is needed as well as RNA repurification to remove buffer, salts, DNase I and digested DNA.

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Guidelines

Additional reagents required:

- Sodium acetate (3 M, pH 5.2)
- Ice-cold 70% ethanol

Please review the Guidelines under Genomic DNA removal and total RNA purification from all types of lysate for important details.

Materials

ISOLATE II Biofluids RNA Kit BIO-52086 by Bioline

Protocol

DNase I Digestion

Step 1.
In a micro centrifuge tube, mix together 2.5 µL of the supplied DNaseI Solution, 10 µL of DNase I Reaction Buffer DRB, and up to 87.5 µL of eluted RNA.

NOTES

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If using a lower starting volume of RNA solution, bring the volume up to 100 µL using RNase-free water.

DNase I Digestion

Step 2.
Gently swirl tube to mix solution.

DNase I Digestion

Step 3.
Gently spin down (approx. 1s at 1000 x g) to collect solution at bottom of tube.

DNase I Digestion

Step 4.
Incubate at room temperature (18-25°C) for 10 min.

DURATION

00:10:00

Ethanol precipitation

Step 5.
Repurify the RNA with a suitable RNA clean-up procedure, e.g. using ethanol precipitation below.

Ethanol precipitation

Step 6.

Add 1/10th volume of sodium acetate (3 M, pH 5.2)

Step 7.
Add between 2.5 and 3 volumes of 96-100% ethanol to one volume of sample. Mix thoroughly.

Ethanol precipitation

Step 8.
Precipitate for one hour at -20°C or overnight at -20°C.

DURATION

01:00:00

NOTES

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Note: Choose longer incubation times if the sample has a low RNA concentration. Shorter incubation times are sufficient for high RNA concentrations.

Ethanol precipitation

Step 9.

Centrifuge at maximum speed for 10 min.

DURATION

00:10:00

Ethanol precipitation

Step 10.

Wash the RNA pellet with ice-cold 70% ethanol.

Ethanol precipitation

Step 11.

Dry the RNA pellet and resuspend the RNA in RNase-free water.