RNX- Plus

Solution for total RNA isolation

For Research Use Only

Product: RNX- Plus Solution
Cat. No. : EX6101

Quantity: 25ml
Store: 2-8°C (For long storage, store at -20°C)

RNX- Plus is a Guanidine / phenol solution for total RNA isolation from homogenized sample. Through the action of Guanidine salt in RNA isolation procedure, simultaneously DNA and protein are precipitated in phenol phase. Aqueous phase contains high quality and all types of the genomic RNA.

Reagents Required But Not Supplied:

Chloroform, Isopropanol, 75% Ethanol, DEPC treated water

Protocol for RNA isolation

- Add 1ml ice cold RNX™ –PLUS solution to 2ml tube containing homogenized sample.
- Vortex 5-10sec. and incubate at room temperature for 5min.
- Add 200µl of Chloroform.
- Mix well for 15sec. by shaking (Do not vortex).
- Incubate on ice or 4°C for 5min.
- Centrifuge at 12000rpm at 4°C for 15min.
- Transfer the Aqueous phase to new RNase-free 1.5ml tube, (do not disturb the mid phase) and add equal volume of Isopropanol.
- Gently mix and incubate on ice for 15 min.
- Centrifuge the mixture at 12000 rpm at 4°C for 15min
- Discard the supernatant and add 1ml of 75% Ethanol, shortly vortex to dislodge the pellet and then centrifuge at 4°C for 8 min. at 7500rpm.
- Discard the supernatant and let the pellet to dry at room temperature for few minutes (do not let dry completely, it will decrease the solubility of the pellet.
- Dissolve pellet in 50µl of DEPC treated water. To help dissolving, place the tube in 55-60°C water bath for 10min.

RNX- Plus solution designed to isolate total RNA from different amounts of biological material.
The obtained RNA is ready for use in all downstream applications like:
RT-PCR, cDNA synthesis, Northern, dot, and slot blot analyses, Primer extension, Poly A^+ RNA selection and etc.
Starting Materials:

- Cell Culture: Up to 1×10^7 cells, depending on the cell line.
- Bacterial cells: Up to 1×10^7 cells
- Tissue: 30-50mg.
- Yeast cell: 5×10^7 cells
- Plant and Filamentous Fungi: Up to 100mg.

- Liquid materials like serum: 100µl
- Whole blood*: At least 500µl (up to 2ml)

*Prior to RNA extraction from whole blood, RBCs must be removed by RBC Lysis Buffer and white blood cells (WBC pellet) must use as starting material.
*Use fresh blood sample for RNA blood extraction.

There are two distinct and essential steps for RNA isolation by RNX- Plus solutions: Disruption and homogenization of sample.
Insufficient disruption and homogenization significantly will reduce RNA yield.

There are different methods:

Sample	Disruption	Homogenization
Cells	RNX solution	Vortex
Tissue	By mortar and pestle liquid Nitrogen	Syringe and Needle
Yeast cell	lyticase	Vortex
Plant and Filamentous Fungi	By mortar and pestle in liquid Nitrogen	Shredder
Liquid materials	RNX solution	Vortex
WBC pellet	RNX solution	Vortex

Precautions:
RNX -Plus contains an irritant (Guanidine thiocyanate) and poison (phenol). Handle with gloves and do not get in eyes, skin, or clothing. Avoid breathing vapor.
In case of contact: Immediately flush eyes or skin with a large amount of water for at least 15 minutes and seek immediate medical attention.

Quality Control:
The concentration of RNA should be determined by measuring the absorbance at 260 nm (A260) in a spectrophotometer. The ratio of the readings at 260nm and 280nm (A260/A280) provides an estimate of the purity of RNA, By RNX- Plus solution the ratio is usually greater than 1.6.

Other related products in SinaClon catalog:
- **RBC Lysis Buffer**, Cat. No.: EX6122
- **DNaseI**,Cat. No.: MO5401
- **DEPC treated Water,** Cat. No.: CH8141
- **DEPC,** Cat. No.: CH8131

TROUBLESHOOTING GUIDE

PROBLEM	CAUSE	SOLUTION
Lower than Expected 260/280	Over dried pellet	Do not use speed vac. Dry pellet briefly at room temperature. During wash step centrifuge at 7500 max. Incubate RNA 10-15 minutes at 60°C.
	Contamination of aqueous layer with interphase /organic phase	Take less aqueous phase. Use small bore pipette tips. Exercise care while removing aqueous layer.
	Sample contains glycogen, polysaccharides or other contaminants	Wash pellet in 4M LiCl prior to ethanol wash.
	Vortexing	Always invert samples during extraction.
Degraded RNA	Trouble with spectrophotometric method	Check spectrophotometric method
	Endogenous RNase Activity Exogenous	Use fresh tissue or cells.
	RNase contamination	

	Homogenization Step extended beyond 20 minutes.	Extract samples within 20 minutes For multiple samples freeze homogenates at –70°C for later simultaneous processing.
DNA Contamination	Contamination of aqueous phase with interphase/organic phase	Take less of aqueous phase. Use small bore pipet tips. Exercise care while removing aqueous phase.

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