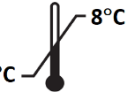
RNX- Plus

(Solution for total RNA isolation)

REF EX6101 Quantity: 25 ml

(For long storage, store at -20°C) RUO



Components

Contents	Amounts
RNX- Plus	25 ml

Description

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 all types of the genomic RNA with high quality.

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

Row	Materials	Amounts
1	Cell Culture	Up to 1×10^7 cells, depending on the cell line
2	Bacterial cell	Up to 1×10^7 cells
3	Tissue	30-50 mg
4	Yeast cell	5×10^7 cells
5	Plant and Filamentous Fungi	Up to 100 mg
6	Liquid materials like serum	100 µl
7	Whole blood	At least 500 µl (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.**

Required materials and tools that are not supplied with the kit:

Row	Materials and Tools	SinaClon Catalog
1	Chloroform	-
2	Isopropanol	-
3	Ethanol 75%	-
4	DEPC treated water	CH8141
5	RBC Lysis Buffer	EX6122
6	DNase I	MO5401
7	DEPC	CH8131

Protocol for RNA isolation

1) Add 1ml ice cold RNX™ – PLUS solution to 2ml tube containing homogenized sample.
2) Vortex 5-10 sec and incubate at room temperature for 5min.
3) Add 200 µl of Chloroform.
4) Mix well for 15 sec by shaking (Do not vortex).
5) Incubate on ice or 4 °C for 5 min.
6) Centrifuge at 12000 rpm at 4 °C for 15 min.
7) Transfer the Aqueous phase to new RNase-free 1.5ml tube, (do not disturb the mid phase) and add equal volume of Isopropanol.
8) Gently mix and incubate on ice for 15 min.
9) Centrifuge the mixture at 12000 rpm at 4 °C for 15 min.
10) Discard the supernatant and add 1 ml of 75% Ethanol, shortly vortex to dislodge the pellet and then centrifuge at 4 °C for 8 min at 7500 rpm.
11) 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.
12) Dissolve pellet in 50 µl of DEPC treated water. To help dissolving, place the tube in 55-60 °C water bath for 10 min.

There are different methods

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.

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 260 nm and 280 nm (A260/A280) provides an estimate of the purity of RNA. By RNX - Plus solution the ratio is usually greater than 1.6.

Signs

Signs	Definitions	Signs	Definitions
	Temperature range on product use		Name and address of the manufacturer of the product
RUO	For Research Use Only	REF	Product technical code

Troubleshooting

This guide may help solve problems that may arise.

PROBLEM	CAUSE	SOLUTION
Lower than Expected 260/280	Over dried pellet	Do not use speed vac. Dry pellet briefly at room temperature.
	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.
	Sample not homogenized with sufficient RNX Reagent.	Use 1 ml RNX Reagent for up to 50 mg tissue or 10^6 cells.
Degraded RNA	Endogenous RNase Activity Exogenous	Use fresh tissue or cells. Process tissue immediately after removal. For cell culture samples, minimize washing steps. Add RNX Reagent directly to plates.
	RNase contamination	
	Improper storage of RNA	Store isolated RNA at -70°C, not -20°C.
	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.
	Insoluble materials were not removed before chloroform extraction.	Remove any particulate material before chloroform addition. This material may trap DNA.

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