SinaPure™ RNA

(Kit for the isolation of RNA from Cell Culture, Animal Tissues, Bacteria, Serum and Plasma)

REF **EX6031**

Σ **50 TESTS**

Room Temperature

RUO

25°C

15°C

Components

Contents	Quantity/ Volume
Spin columns	50PCS
Collection tubes	50PCS
Lysis solution	20ml
Precipitation solution	15ml
Wash I solution	30ml
Wash II solution	50ml
RNase free water	2×1250µl

Description

SinaPureTM – RNA kit system is one of the latest nucleic acid purification technologies. This kit contains all ingredients for quick preparation of RNA from tissues, cell cultures, bacteria and Plasma. It presents remarkable features of timesaving, easy, prompt and high yield RNA purification. Basis of the technology is the binding of RNA to matrices including silica membrane filters in presences of high salts concentration and reversibly elution in low salt condition like RNase free water. Obtained RNA is suitable for downstream applications including RT PCR, cDNA synthesis. This kit contains a manual with detailed protocols of RNA extraction.

Storage and Stability

Spin columns and the kit content are packed in closed bags and can be stored at room temperature (15-25°C) for at least 1 year. Care should be taken while using, to keep the hygiene state of the columns once opened. For long storage, 2-8 °C is recommended.

Important notes: please read before starting

All centrifugation steps should be done at room temperature (15-25°C). Warm Lysis buffer by placing in 37 °C for 15min and then shake softly. Set heater block or water bath at 55°C. You need a bench top micro centrifuge (12,100g, 13,000rpm), precision pipettes and sterile pipette tips allowing pipetting volumes 1 to 10µl, up to 100µl and up to 1000µl, and sterile 1.5ml or 2ml polypropylene tubes.

Warnings and Precautions

Lysis and wash I Buffer are toxic and irritant. They should be open in fume hood. Avoid contact any kit reagents with skin & eyes. Wear gloves before using SinaPure™ RNA and changed it frequently during RNA purification, skin is a common source of RNases. Lysis and wash I Solution are toxic and irritant and they should be open in fume hood. Contact of Lysis and wash I buffer Solutions with acids or bleach solution, liberate toxic gas. When handling biological samples, follow recommended procedures for biohazardous materials.

Protocol

A. Sample Preparation

1. Cell Culture:

Depending on the cell line in round-bottomed 2ml tubes, collect up to $1x10^7$ cells by centrifugation, 5min at 3000RPM. In general, $4\text{-}6x10^6$ cells may need to obtain optimal RNA yield. Discard supernatant completely by pipetting to remove residual growth medium. Rinse the cell pellet by PBS and repeat the centrifugation step. Remove supernatant completely by pipetting. Dislodge cell pellet by gentle finger tapping then add 400µl Lysis solution. Disrupt and homogenize cells by vortexing in one minute. Incomplete homogenization leads to significantly reduced RNA yields.

Follow the protocol.

2. Tissues

Cut 25-50mg (for RNA active tissue 10mg) fresh tissues. Grind it by mortar and pestle in liquid nitrogen. Transfer tissues powder to round-bottomed 2ml tube contains 400µl Lysis solution immediately. Mix it thoroughly by vortexing for one minute*.

*Higher yield of RNA may achieve by Rotor–stator homogenizer for tissue Lyser.

Follow the protocol.

3. Bacterial cultures

In 2ml tube, Collect 1x10^9 bacterial cells by centrifugation 2min at 10000RPM. Discard supernatant completely by pipetting to remove residual growth media. Resuspend the pellet in 150µl fresh prepared Lysozyme (0.4mg/ml TE, not provided). Invert the tube several times, do not vortex it. Incubate at room temperature for 5 minutes. Add 400 µl Lysis solution. Mix it thoroughly by vortexing for 20 seconds.

Follow the protocol.

4. Sera/ Plasma

Add 100µl serum or plasma to 1.5ml micro centrifuge tube contains 400µl Lysis solution. Mix it thoroughly by vortexing for 20 seconds.

Follow the protocol.

For long-term storage the samples should be aliquoted or stored in needed portions and kept at -20°C or colder -70°C. To avoid any nuclease activity, keep samples frozen until RNA extraction. Therefore, add pre warm Lysis buffer to frozen samples and shake softly to complete thawing and follow the protocol.

B. Extraction Protocol

1. Add 300µl Precipitation solution, close the lid and invert it for ten times.
2. Transfer the solution to a spin column with collection tube (included) by pipetting.
3. Centrifuge the tube at (12,100g, 13,000rpm) for 1 min. Discard flow-through. (1) *
4. Add 300µl Wash I Solution to spin column. Centrifuge at (12,100g, 13,000rpm) for 1 min. Discard flow-through.
5. Add 300µl of Wash I Solution to spin column and centrifuge at (12,100g, 13,000rpm) for 1 min. Discard flow-through.

6. Add 500µl of Wash II Solution to spin column and centrifuge at (12,100g, 13,000rpm) for 1 min. Discard flow-through.
7. Add 500µl of Wash II Solution to spin column and centrifuge at (12,100g, 13,000rpm) for 1 min. Discard flow-through.
8. Centrifuge at (12,100g, 13,000rpm) for 3 min. (2) *
9. Carefully transfer the column to a new 1.5ml tube (not included).
10. Place 50µl RNase free water in the center of the column, close lid and incubate for 5-10 min at room temperature. Thereafter, centrifuge at (12,100g, 13,000rpm) for 1 min to elute the RNA. (3) *

(1) *	- You can discard collection tube and place column in new 2 ml tube (not included).
(2) *	- Avoid contaminating the column with ethanol. Ensure that the column is dry, and no ethanol contaminates the tip of the column. If you observe residues of ethanol, place the column in a new reaction tube.
(3) *	The elution volume depends on the sample: - If high RNA amounts are expected, final elution volume may increase up to 200µl. - High concentrated RNA may achieve when elution volume reduced to 30- 50µl. - 30µl RNase free water would be sufficient for 100µl serum or plasma samples. In general, 50-100µl RNase free water volume gives satisfactory results. An alternative way of increasing the RNA yield is repeated centrifugation. Transfer eluted RNA to center of membrane filter again and centrifuge at (12,100g, 13,000rpm) for 1 min to increase the RNA yield.

Quality Control

A.RNA Quality Control

Determination of RNA concentration is done by UV reading at 260nm. RNA preparations should be vortexed shortly and diluted (if needed) accordingly by 10mM Tris-HCl, pH 8.0 or RNase free water. Blank and dilution buffer / water should be the same. A standard procedure of measuring RNA quality is the determination of the absorption quotient (Q) of readings at A260nm and A280nm: Q = A260nm/A280nm. For a pure RNA preparation, Q lies between 1.7 and 2.0.
Denaturing gel electrophoresis and staining by ethidium bromide may also be used to determine quality of purified RNA.
In general, approximate ratio between 28S rRNA to 18S RNA should be 2:1.
Since Viral RNA from sera samples quantity is too small, it is invisible in agarose gel and does not detectable by spectrophotometer.
DNA may remain in final eluted RNA and after purification. If complete removal of DNA is required, RNA should be treated by DNase I.

Removal of DNA (Optional, not provided)

When DNA-free genomic RNA is required, DNase I should be added to the sample. In order to do such please see Deoxyribonuclease I protocol (Ref/Cat. No.: MO5401).

B. Kit quality control

All components of kit are successfully tested in the RNA purification from CHO Frozen, fresh cells or E. Coli bacterial pellet. All were controlled by cDNA synthesis and followed by qRT PCR.

Signs

Signs	Definitions
	Temperature range on product use
	For Research Use Only
	Product shipping conditions
	Name and address of the manufacturer of the product
	Product technical code
	Number of usable tests

Troubleshooting

This guide may help solve problems that may arise.

Observation	Possible cause	Comments/suggestions
Low or no RNA yield	Inefficient lysis of sample	- Make sure that homogenization step by vortex were enough. - Extend homogenization step. - pass lysate through 20- Gauge needle at least for 10 times. - Optimize amount of starting material.
	Sample was frozen and thawed several times.	- Keep samples frozen until RNA extraction. Whenever possible, fresh samples should be used and processed immediately. Storage samples at 20°C or colder -70°C / liquid nitrogen or RNA Stabilization reagent immediately after harvesting. Several Freeze & thaw cycles should be avoided. - Take new sample.
	Filter may be clogged during purification	- Check Lysis solution for any crystal formation. - Warm lysis before purification. -Check lysate for any tissues or particle remaining. Remove particles by centrifuge for 5 mint/ 12000g and transfer supernatant to a new tube. - Extend homogenization step.
Degraded RNA	RNase contamination	- Keep samples frozen until RNA extraction. - Whenever possible, fresh samples should be used and processed immediately. Storage samples at -20°C or colder -70°C / liquid nitrogen or RNA Stabilization reagent immediately after harvesting. - Freeze & thaw cycles should be avoided. - Wear gloves during all procedure and change it frequently. - Use only sterilized and RNase-free glass and plastic ware in order to avoid RNase Contamination.
DNA contamination	No DNase treatment	Digest RNA preparation with DNase I.
No enzymatic reaction (in case of using enzymes)	Residues of ethanol	- Before adding Elution buffer ensure that the column is dry, and no ethanol contaminates the tip of the column. If you observe residues of ethanol, place the column in a new reaction tube. Centrifuge again at (12,100g, 13,000rpm) for 1min more.

شکایات مشتری

SinaClon BioScience شرکت سیناکلون

نظرسنجی از مشتری

Unit 9, Rouyesh building, Science and Technology Park, Tarbiat Modares University, Pajouhesh Blvd, Tehran, Iran

+982191082111

hi@sinaclon.com

www.sinaclon.com