SinaPure™ RNA

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

For research Use Only

Cat. No.: EX6031 **50 Preparations**

Store kit contents at Room Temperature

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SinaPure™ RNA

Kit for the isolation of RNA from Cell culture, Tissues, Bacteria and Plasma.

EX6031 50 Preps Store at RT

Assembled mini spin columns	50x
Collection tubes	50x
Lysis Buffer	20 ml
Precipitation Buffer	15 ml
Wash Buffer I	20 ml
Wash Buffer II	40 ml
RNase free water	2x1250µl

Warning and precautions

Avoid contact any kit reagents with skin & eyes. Wear gloves and changed it frequently during RNA purification, skin is a common source of RNases. Lysis Buffer and Wash Buffer 1 both are irritants. Contact of Lysis Solution with acids or bleach solution, liberates toxic gas. Follow recommended procedures for biohazardous materials when handling human samples.

Storage and Stability

Spin columns of the kit show full performance in at room temperature (18-25°C) for at least one year. Please take care that columns, once opened, should be used instantly. Close bottles immediately after use. For long storage, 2-8°C is recommended.

Kit Description

This kit contains all ingredients for quick preparation of RNA from tissues, cell cultures, bacteria and Plasma. The kit contains spin columns, buffers and reagents necessary for lysis of material, RNA binding to the matrix, and washing and elution of RNA into small volume from the matrix. Each kit contains a manual with detailed protocols of RNA extraction.

SinaPure™ –RNA kit system is one of the latest nucleic acid purification technologies. This kit 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...

Important notes, please read before starting

Lab temperature and all centrifugation steps should be down at 18- 25°C.

Warm Lysis buffer by placing in 37°C for 15 min and finally softly shake. Heat a water bath or heater block to 55°C. You need a bench top micro centrifuge (12000g, 13000RPM), precision pipettes and sterile pipette tips allowing pipetting volumes 1 to 10 µl, up to 100 µl and up to 1000 µl, and sterile 1.5 ml or 2 ml polypropylene tubes. Place spin columns in a rack before starting with the extraction protocol. Open spin columns only before use.

WARNINGS AND PRECAUTIONS

Caution: *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 use SinaPure™ RNA. 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.

This kit is designed for total RNA extraction from mammalian cells, tissue, Bacterial cultures and Plasma.

All steps perform at Room temperature.

Sample Preparation

- Cell Culture

Depending on the cell line in round-bottomed 2 ml tubes, collect up to 1 x 10^7 cells by centrifugation, 5 min at 3000RPM.

In general 4-6x10^6 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. Dislodged cell pellet by soft hitting then add 400 µl Lysis solution.
Disrupt and homogenize cells by vortexing in one minute. For sufficient homogenization, pass lysate through 20- Gauge needle at least for ten times. Incomplete homogenization leads to significantly reduced RNA yields. Follow the protocol.

-Tissues

Cut 25-50 mg (for RNA active tissue 10mg) fresh tissues. Grind it by mortar and pestle in liquid nitrogen. Transfer tissues powder to round-bottomed 2 ml tube contains 400 µl Lysis solution immediately. Mix it thoroughly by vortexing for one minute. For homogenization pass lysate through 20- Gauge needle at least for ten times*. Follow the laboratory protocol.
*Higher yield of RNA may achieve by Rotor–stator homogenizer or tissue Lyser.

- Bacterial cultures

In 2 ml tube, Collect 1x10^9 bacterial cells by centrifugation 2 min at 10000RPM. Discard supernatant completely by pipetting to remove residual growth media. Resuspend the pellet in 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 of Lysis solution. Follow the laboratory protocol.

-Sera/ Plasma

Add 100µl serum or plasma in 1.5 ml micro centrifuge tube contains 400 µl Lysis solution immediately. Mix it thoroughly by vortexing for 20 seconds. Follow the laboratory 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 avoiding any nuclease activity keep samples freeze until RNA extraction. Therefore add pre warm Lysis buffer to freeze samples and softly shake to complete defreezing and follow the protocol.

Protocol:

Approximate time for total RNA purification is about ≠ 15 min

2- Add 300 µl Precipitation solution, close the lid and invert it for ten times.

3- Transfer the solution to a spin column with collection tube (included) by pipetting.

4- Centrifuge the tube at (12000x g, 13000RPM) for 1 min. Discard flow-through.*

5-Add 400 µl Wash buffer I to spin column. Centrifuge at 12000g(13000RPM) for 1 min. Discard flow-through.

6- Wash the spin column with 400 µl of Wash buffer II centrifugation at 12000g (13000RPM) for 1 min. Discard flow-through.

7- Wash the spin column with 400 µl of Wash buffer II by centrifugation at 12000g (13000RPM) for 1 min. Discard flow-through.

8- Centrifuge at 12000g(13000RPM) for 2 min.**

9-Carefully transfer the column to a new 2ml ml tube (not included).
Place 50 µl 55°C pre heated RNase free water in the center of the column, close lid and incubate for 3-5 min at 55°C. Thereafter, centrifuge at 12000g (13000RPM) for 1 min to elute the RNA.***

*You can discard collection tube and place column in new 2 ml tube (not included).

**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.

*** The elution volume depends on the sample:

- If high RNA amounts are expected, final elution volume may increase up to 200ul.
- High concentrated RNA may achieve when elution volume reduced to 30- 50ul.
- 30 µl RNase free water would be sufficient for 100 µl 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 12000g (13000RPM) for 1 min to increase the RNA yield.

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 and disruption steps were enough. Extend both steps. Use sufficient starting materials. Decrease it, if more than indicated samples used.
-	In sufficient sample storage	Keep samples freezed 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. Take new sample.
-	Filter may 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. Use sufficient starting materials. Decrease it, if more than indicated samples used. Centrifugation temperature too low. The centrifugation temperature should be 20–25°C.
Degraded RNA	RNase activity	Starting materials stored or handled incorrectly: Keep samples freezed 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. RNase contamination. 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	Residues of ethanol	Before adding RNase free water 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 12000g (13000 RPM) for 1 min more.

RNA quality control

Determination of RNA concentration is done by UV reading at 260 nm. RNA preparations should be vortexed shortly and diluted (if needed) accordingly by 10 mM 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. For complete remove of any trace and remaining DNA, DNasI treatment should be perform:

Add to an RNase-free tube: RNA 1 µg
10X reaction buffer with MgCl2: 1 µl
DNase I, RNase-free:1 µl (1 u)
DEPC-treated Water: to 10 µl
Incubate at 37°C for 30 min.

Add 1 µl 50 mM EDTA and incubate at 65°C for 10 min to inactivate the DNase I. Addition of EDTA is required as RNA hydrolyzes during heating with divalent cations in the absence of a chelating agent.

Kit quality control:

$4x10^6$ CHO Freeze or fresh cells lysed and homogenized according to above protocol. The purified RNA has an A260/280 ratio of ≥1.9 and successfully tested in cDNA synthesis followed by qRT.

With this product you may need to:
- **DNaseI:** MO5401
- **DEPC:** CH8131
-**DEPC treated Water:** CH8141
-**Lysozyme:** MO5471

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