SinaPure™ ONE

(Kit for simultaneous isolation of genomic DNA and total RNA from the same sample)

EX6051

50 TESTS

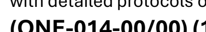
Room Temperature

Components

Contents	Quantity/ Volume	Store Temperature
Mini spin columns	50X	RT
Collection tubes (1.5ml)	50X	RT
Lysis Buffer	20ml	RT
Precipitation Buffer	15ml	RT
Wash Buffer I	20ml	RT
Wash Buffer II	40ml	RT
Elution Buffer	5ml	RT

Description

SinaPure™ – ONE is one of the latest nucleic acid's purification technologies. This kit is designed for simultaneous and rapid purification of DNA/ RNA from the biological samples. It presents remarkable features of timesaving, easy, prompt and high yield nucleic acids (NA) purification. Basis of the technology is the binding of NA to matrices including DNase & RNase free silica membrane filters in presences of high salts concentration and reversibly elution in low salt condition like Nuclease free elution water or 10mM Tris-HCl. Obtained NA is suitable for PCR and cDNA synthesis. It contains all ingredients for quick preparation of pure NA from biological samples like: serum, plasma, cell cultures, bacterial pellet and tissues. This kit contains a manual with detailed protocols of DNA 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.

Important notes: please read before starting

Warm Lysis buffer by placing in 37 °C for 15min and then shake softly. Set heater block 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. All centrifugation steps should be done at room temperature (15-25°C).

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™ ONE. 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 NA 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 NA 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 NA 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. Dislodge cell pellet by gentle finger tapping then add 400 µl Lysis solution. Disrupt and homogenize cells by vortexing in one minute. Incomplete homogenization leads to significantly reduced NA yields.
Follow the protocol.

4. Sera/ Plasma

Add 100µl serum or plasma, body fluid or virus-infected cell culture supernatant to 1.5ml micro centrifuge tube contains 400µl Lysis solution. Mix it thoroughly by vortexing for 20 seconds.
Follow the protocol.

5. Buccal or Vaginal Swab

Place swab in 1.5ml micro centrifuge tube, contains 400µl lysis solution. Close the tube and vortex vigorously. Press the stem end of swab towards the tube two to three times and then remove swab.
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 NA 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 and vortex at max speed for 5 seconds.
2. Transfer the solution to a spin column with collection tube (included) by pipetting.
3. Centrifuge the tube at (12,100g, 13,000rpm) for 1min. Discard flow-through. (1)*
4. Place spin column in collection tube, add 400µl Wash buffer I to spin column. Centrifuge at (12,100g, 13,000rpm) for 1min. Discard flow-through.
5. Wash the spin column with 400µl of Wash buffer II centrifugation at (12,100g, 13,000rpm) for 1min. Discard flow-through.
6. Wash the spin column with 400µl of Wash buffer II by centrifugation at (12,100g, 13,000rpm) for 1min. Discard flow-through.
7. Place spin column in collection tube. Centrifuge at (12,100g, 13,000rpm) for 2min. (2) *
8. Carefully transfer the column to a new 1.5ml tube (not included). Place 50µl of 55°C pre-heated elution buffer in the center of the column, close lid and incubate for 3-5min at 55 °C. Thereafter, centrifuge at (12,100g, 13,000rpm) for 1min to elute the NUCLEIC ACID, (NA). (3) *

(1) *	- You can discard collection tube and place column in new 2ml 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 NA amounts are expected, a higher elution volume may increase the NA yield. Generally, 50-100µl elution volume gives satisfactory results. - An alternative way of increasing the NUCLEIC ACID, (NA) yield is repeated centrifugation. Transfer eluted NA to center of membrane filter again and centrifuge at (12,100 g, 13,000 rpm) for 1min to increase the NA yield. - 30µl Nuclease free elution buffer would be sufficient for 100 µl serum or plasma samples.

With this kit you are able to co purify both DNA and RNA in a single sample. Although RNA may inhibit some downstream enzymatic reactions but it does not affect PCR so eluted NA from serum, plasma or virus-infected cell culture supernatant can be used directly in PCR reaction.
If complete removal of DNA or RNA is required, NA should be treated by DNase I or RNase A respectively.

C. 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).

D. Removal of RNA (Optional, not provided)

When RNA-free DNA is required, RNase A should be added to the sample. In order to do such please see Ribonuclease A protocol (Ref/Cat. No.: MO5411).

Quality Control

A. Nucleic Acid Quality Control

Agarose gel electrophoresis of prepared NA is a direct method for testing obtained NA quality in terms of molecular size and conformation. Depending on the expected amount, pipette 5-10µl eluted NA directly to a gel slot.
For samples such as serum, plasma or virus-infected cell culture supernatant, obtained NA is invisible in agarose gel and not detectable by spectrophotometer.
Evaluate and prove amplifiable quality of extracted DNA by amplification of a housekeeping gene.

B. DNA Quality Control

RNase A treatment should be done before determination of DNA concentration by UV.
Pure DNA preparations should be vortexed shortly and diluted (if needed) accordingly by 10mM Tris-HCl or elution buffer. Blank and dilution buffer should be the same. A standard procedure of measuring DNA quality is the determination of the absorption quotient (Q) of readings at A260nm and A280nm:
Q = A260nm /A280nm. For a pure DNA preparation, Q lies between 1.7 and 2.0.

C. RNA Quality Control

Determination of RNA concentration is done by UV reading at 260 nm. It should be done after DNase I treatment. 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.

D. Kit Quality Control

All components of this Kit are successfully tested in the NA purification from E. Coli bacterial pellet, Rat, human tissue and buccal swab. All were controlled by cDNA synthesis and followed by amplification reaction for mtlD and GAPDH genes respectively.

Signs

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

Troubleshooting

This guide may help to solve problems that may arise.

Observation	Possible cause	Comments/suggestions
Low or no NA 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 NA extraction. Whenever possible, fresh samples should be used and processed immediately. Several freeze-thaw cycles should be avoided. - Take new sample.
	Filter may clogged during purification	- Check Lysis solution for any crystal formation. - Check lysate for any tissues or particle remaining. - Warm lysis before purification - Extend homogenization step or remove particles.
Degraded RNA	RNase contamination	- Keep samples frozen until RNA extraction. - Whenever possible, fresh samples should be used and processed immediately. Store 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.
Low NA quality	Salt in elute	- Repeat washing steps (I and II)
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, 13,000 rpm) for 1min more.

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