Tehran Cavosh Clon
Our Aim is Your Success

SinaPure™ ONE

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

For Research Use Only

Cat. No: EX٦٠٥١ **٥٠ Preparations**

Store kit contents at:٢-٨°C **Store DNase I at: -٢٠°C**

SinaClonBioScience

www.sinaclon.com
Central Office: No. ٥٦, Azimi St., Nafisi Ave., Phase ١, Ekbatan, Tehran, Iran, ١٣٩٣٨٣٣١٦١
Tel: +٩٨(٠)٢١ ٤٤٦٣ ٠٠٥٠-٥١
+٩٨(٠)٢١ ٤٤٦٦ ٥١٥٦
Customer Service:
+٩٨(٠)٢١ ٤٤٦٣ ٣٠١٦
+٩٨(٠)٩٠٢٣١٢٠٠٥٩
Place your order: **order@sinaclon.com**

SinaPure™ ONE

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

EX٦٠٥١ ٥٠ Preps Store at ٢-٨°C , Store DNaseI at -٢٠°C

Note: please keep Lysis Buffer, mini spin columns and collection tubes at room temperature

Kit content

Mini spin columns	**٥٠x**
Collection tubes (١,٥ ml)	**٥٠x**
DNaseI set	**١x**
Lysis Buffer	**٢٠ ml**
Precipitation Buffer	**١٥ ml**
Wash Buffer I	**٢٠ ml**
Wash Buffer II	**٤٠ ml**
Nuclease free water	**٥ml**

Storage and Stability

Spin columns of the kit are packed in closed bags and show full performance in this state at room temperature (١٨-٢٥°C) for at least ١ year. DNaseI is delivered as a solution and should be stored upon arrival at -٢٠ °C. This guarantees performance for ٢ year. For long storage and to avoid any probable contamination, keep Elution Buffer at ٢-٨°C.
Please take care that columns, once opened, should be used instantly. Close bottles immediately after use.

Kit Description

This kit designed for simultaneous and rapid purification of DNA/ RNA from the single biological samples. It contains all ingredients for quick preparation of pure nucleic acids (NA) from biological samples like: serum, plasma, cell cultures, bacterial pellet and tissues. The kit contains spin columns, buffers and reagents necessary for lysis of material, nucleic acids binding to the matrix, washing and elution of NA into small volume from the matrix. Each kit contains a manual with detailed protocols of DNA extraction.
SinaPure™ –ONE is one of the latest nucleic acids purification technologies. This kit presents remarkable features of timesaving, easy, prompt and high yield 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 ١٠mM Tris-HCl.
Obtained NA is suitable for PCR and cDNA synthesis.

Important notes: please read before starting

Warm Lysis buffer by placing in ٣٧°C for ١٥ min and finally softly shake. Heat heater block to ٥٥°C. You need a bench top micro centrifuge (١٢,١٠٠ x g, ١٣,٠٠٠ rpm), precision pipettes and sterile pipette tips allowing pipetting volumes ١ to ١٠ µl, up to ١٠٠ µl and up to ١٠٠٠µl, and sterile ١,٥ ml or ٢ ml polypropylene tubes. Place spin columns in a rack before starting with the extraction protocol. Open spin columns only directly before use.
All centrifugation steps should be done at room temperature (١٨-٢٥°C).

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™ 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.

Sample Preparation

- Cell Culture

Depending on the cell line in round-bottomed ٢ ml tubes**,** collect up to ١ x ١٠ ^٧cells by centrifugation, ٥ min at ٣٠٠٠RPM. In general ٤-٦x١٠^٦ 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. Dislodged cell pellet by soft hitting then add ٤٠٠ µl Lysis solution.
Disrupt and homogenize cells by vortexing in one minute. For sufficient homogenization, pass lysate through ٢٠- Gauge needle at least for ten times. Incomplete homogenization leads to significantly reduced NA yields.
Follow the protocol.

-Tissues

Cut ٢٥-٥٠ mg (for RNA active tissue ١٠mg) fresh tissues. Grind it by mortar and pestle in liquid nitrogen. Transfer tissues powder to round-bottomed ٢ ml tube contains ٤٠٠ µl Lysis solution immediately. Mix it thoroughly by vortexing for one minute. For homogenization pass lysate through ٢٠- Gauge needle at least for ten times*. Follow the laboratory protocol.
*Higher yield of NA may achieve by Rotor–stator homogenizer or tissue Lyser.

- Bacterial cultures

In ٢ ml tube, Collect ١x١٠^٩ bacterial cells by centrifugation ٢ min at ١٠٠٠٠RPM**.** Discard supernatant completely by pipetting to remove residual growth media. Dislodged cell pellet by soft hitting then add ٤٠٠ µl Lysis solution.
Disrupt and homogenize cells by vortexing in one minute. For sufficient homogenization, pass lysate through ٢٠- Gauge needle at least for ten times. Incomplete homogenization leads to significantly reduced NA yields.
Follow the protocol.

-Sera/ Plasma

Add ١٠٠µl serum or plasma, body fluid or virus-infected cell culture supernatant to 1.5 ml micro centrifuge tube contains ٤٠٠ µl Lysis solution. Mix it thoroughly by vortexing for ٢٠ seconds. Follow the protocol.

-Buccal or Vaginal Swab

Place swab in ١,٥ml micro centrifuge tube, contains ٤٠٠ µl lysis solution. Close the tube and vortex vigorously. Press the stem end of tube towards the swab 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 avoiding any nuclease activity keep samples freeze until NA extraction. Therefore add pre warm Lysis buffer to freeze samples and softly shake to complete thawing and follow the protocol.

Protocol

***Approximate time for total nucleic acid preparation≠١٥* min.**

١. Add ٣٠٠ µl Precipitation solution and vortex at max speed for ٥ seconds.
٢. Transfer the solution to a spin column with collection tube (included) by pipetting.
٣. Centrifuge the tube at (١٢,١٠٠ x g, ١٣,٠٠٠ rpm) for ١ min. Discard flow-through.*
٤. Place spin column in collection tube, add ٤٠٠ µl Wash buffer I to spin column. Centrifuge at ١٢,١٠٠ x g (١٣,٠٠٠ rpm) for ١ min. Discard flow-through.

٥. Wash the spin column with ٤٠٠ µl of Wash buffer II centrifugation at ١٢,١٠٠ x g (١٣,٠٠٠ rpm) for ١ min. Discard flow-through.

٦. Wash the spin column with ٤٠٠ µl of Wash buffer II by centrifugation at ١٢,١٠٠ x g (١٣,٠٠٠ rpm) for ١ min. Discard flow-through.

٧. Place spin column in collection tube. Centrifuge at ١٢,١٠٠ x g (١٣,٠٠٠ rpm) for ٢ min.**

٨. Carefully transfer the column to a new ١,٥ ml tube(not included). Place ٥٠µl ٥٥°C pre heated elution buffer in the center of the column, close lid and incubate for ٣-٥ min at ٥٥°C. Thereafter, centrifuge at ١٢,١٠٠ xg (١٣,٠٠٠ rpm) for ١ min to elute the NUCLEIC ACID, (NA).***

*You can discard collection tube and place column in new ٢ 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 NA amounts are expected, a higher elution volume may increase the NA yield. Generally, ٥٠-١٠٠µl elution volume gives satisfactory results.

- An alternative way of increasing the NUCLEIC ACID, (NA) yield is repeated centrifugation. Transfer ٤٥-٩٥ ul eluted NA to center of membrane filter again and centrifuge at ١٢,١٠٠ x g (١٣,٠٠٠ rpm) for ١ min to increase the NA yield.
- ٣٠ µl Nuclease free elution buffer would be sufficient for ١٠٠ 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 DNaseI or RNase A respectively.
Depends to your application divide obtained NA in two separate tubes. Estimate NA concentration at ٢٦٠nm wavelength and follow the protocol:

Removal of DNA (Provided):
When DNA-free genomic RNA is required, DNase I should be added to the sample, as indicated in the protocol:

Add to an DNA-free tube

Nucleic acid	١ µg
١٠X reaction buffer with MgCl٢	١ µl
DNase I, RNase-free:	٠,٥ µl/ ٠,٥ u
Nuclease free water :	to ١٠ µl

Incubate at ٣٧°C for ٣٠ min.

Add ١ µl ٥٠ mM EDTA and incubate at ٦٥°C for ١٠ 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.

Removal of RNA (Optional, not provided):
RNase A cleaves single-stranded and double-stranded RNA. The working concentration of RNase A is ١-١٠٠µg/ml, depending on the application.
Incubate NA at ٣٧°C for ٣٠ minutes.
This enzyme reliably removed by spin column or phenol/chloroform extraction.

Troubleshooting
This guide may help 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. - Optimize starting materials. Use the recommended amount of starting material - Extend homogenization step.
-	Sample was frozen and thawed several times.	Keep samples freezed 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 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 -٢٠ °C or colder -٧٠°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.
Low NA quality	Salt in elute	Repeat washing steps (I and II)
No enzymatic reaction	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 ١٢,١٠٠ x g (١٣,٠٠٠ rpm) for ١ min more**

Nucleic Acid Quality Control

Agarose gel electrophoresis of prepared NA isa direct method for testing obtained NA quality in terms of molecular size and conformation. Depending on the expected amount, pipette ٥-١٠ul 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.

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 ١٠ mM 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 A٢٦٠nm and A٢٨٠nm:

Q = A٢٦٠nm /A٢٨٠nm. For a pure DNA preparation, Q lies between ١,٧ and ٢,٠.

RNA Quality Control

Determination of RNA concentration is done by UV reading at ٢٦٠ nm. It should be done after DNase I treatment. RNA preparations should be vortexed shortly and diluted (if needed) accordingly by ١٠ mM Tris-HCl, pH ٨,٠ 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 A٢٦٠nm and A٢٨٠nm:

Q = A٢٦٠nm /A٢٨٠nm. For a pure RNA preparation, Q lies between ١,٧ and ٢,٠.

Denaturing gel electrophoresis and staining by ethidium bromide may also be used to determine quality of purified RNA.

In general approximate ratio between ٢٨S rRNA to ١٨S RNA should be ٢:١.

Since Viral RNA from sera samples quantity is too small, it is invisible in agarose gel and does not detectable by spectrophotometer.

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.

SinaClon BioScience

www.sinaclon.com
Central Office: No. ٥٦, Azimi St., Nafisi Ave., Phase ١, Ekbatan, Tehran, Iran, ١٣٩٣٨٣٣١٦١
Tel: +٩٨(٠)٢١ ٤٤٦٣ ٠٠٥٠-٥١
+٩٨(٠)٢١ ٤٤٦٦ ٥١٥٦
Customer Service:
+٩٨(٠)٢١ ٤٤٦٣ ٣٠١٦
+٩٨(٠)٩٠٢٣١٢٠٠٥٩
Place your order: **order@sinaclon.com**