



CinnaPure ONE

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

Cat. No. PR911684

50 Preparations

Store kit contents at: 2-8°C

Store DNaseI at -20°C

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CinnaGen: Pioneer in Molecular Biology and Biotechnology

CinnaPure ONE

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

PR911684 50 Preps Store at 2-8°C, Store DNase I at -20°C

Kit content

Mini spin columns	50x
Collection tubes (1.5 ml)	50x
DNaseI set	1x
Lysis Buffer	20 ml
Precipitation Buffer	15 ml
Wash Buffer I	20 ml
Wash Buffer II	40 ml
Nuclease free water	5ml

Storage and Stability

Spin columns of the kit are packed in closed bags and show full performance in this state at room temperature (18-25°C) for at least 1 year. DNaseI is delivered as a solution and should be stored upon arrival at -20 °C. This guarantees performance for 2 year. For long storage and to avoid any probable contamination, keep Elution Buffer at 2-8°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 NA extraction.

CinnaPure –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 10mM Tris-HCl.

Obtained NA is suitable for PCR and cDNA synthesis.

Important notes: please read before starting

Warm Lysis buffer by placing in 37°C for 15 min and finally softly shake. Heat heater block to 55°C. You need a bench top micro centrifuge (12.100 x g, 13.000 rpm), precision pipettes and sterile pipette tips allowing pipetting volumes 1 to 10 µl, up to 100 µl and up to 1000µl, 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 directly before use.

All centrifugation steps should be done at room temperature (18-25°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 CinnaPure 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 2 ml tubes, collect up to 1×10^7 cells by centrifugation, 5 min at 3000RPM.

In general $4-6 \times 10^6$ 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. 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 NA 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 NA may achieve by Rotor–stator homogenizer or tissue Lyser.

- Bacterial cultures

In 2 ml tube, Collect 1×10^9 bacterial cells by centrifugation 2 min at 10000RPM. Discard supernatant completely by pipetting to remove residual growth media. 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 NA yields.

Follow the protocol.

-Sera/ Plasma

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

-Buccal or Vaginal Swab

Place swab in 1.5ml micro centrifuge tube, contains 400 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 ≈ 15 min.

1. Add 300 µl 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 (12.100 x g, 13.000 rpm) for 1 min. Discard collection tube.
4. Place spin column in collection tube, add 400 µl Wash buffer I to spin column. Centrifuge at 12.100 x g (13.000 rpm) for 1 min. Discard flow-through.
5. Wash the spin column with 400 µl of Wash buffer II centrifugation at 12.100 x g (13.000 rpm) for 1 min. Discard flow-through.
6. Wash the spin column with 400 µl of Wash buffer II by centrifugation at 12.100 x g (13.000 rpm) for 1 min. Discard flow-through.
7. Place spin column in collection tube. Centrifuge at 12.100 x g (13.000 rpm) for 2 min.*
8. Carefully transfer the column to a new 1.5 ml tube (not included). Place 50** µl 55°C pre heated elution buffer in the center of the column, close lid and incubate for 3-5 min at 55°C. Thereafter, centrifuge at 12.100 xg (13.000 rpm) for 1 min to elute the NUCLEIC ACID, (NA).**

* 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, 50-100 µl elution volume gives satisfactory results.

- An alternative way of increasing the NUCLEIC ACID, (NA) yield is repeated centrifugation. Transfer 45-95 µl eluted NA to center of membrane filter again and centrifuge at 12.100 xg (13.000 rpm) for 1 min to increase the NA yield.
- 30 µl 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 DNaseI or RNase A respectively.

Depends to your application divide obtained NA in two separate tubes. Estimate NA concentration at 260nm 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	1 µg
10X reaction buffer with MgCl ₂	1 µl
DNase I, RNase-free:	0.5 µl/ 0.5 u
Nuclease free 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.

Removal of RNA (Optional, not provided):

RNase A cleaves single-stranded and double-stranded RNA. The working concentration of RNase A is 1-100µg/ml, depending on the application.

Incubate NA at 37°C for 30 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 -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.

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 12.100 x g (13.000 rpm) for 1 min more

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 5-10ul 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 10 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 A260nm and A280nm:

$Q = A_{260nm} / A_{280nm}$. For a pure DNA preparation, Q lies between 1.7 and 2.0.

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 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:

$Q = A_{260nm} / A_{280nm}$. 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.

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 mtID and GAPDH genes respectively.

