SinaPure™ Viral

Kit for simultaneous isolation of viral DNA and RNA

For Research Use Only

Cat. No.:EX6061 **50 Preparations**

Store kit contents at: 2-8°C

SinaClonBioScience

www.sinaclon.com
Central Office: No. 56, Azimi St., Nafisi Ave., Phase 1, Ekbatan, Tehran, Iran, 1393833161
Tel: +98(0)21 4463 0050-51
+98(0)21 4466 5156
Customer Service:
+98(0)21 4463 3016
+98(0)9023120059
Place your order: order@sinaclon.com

SinaPure™Viral, Kit for simultaneous isolation of viral DNA and RNA from the same sample.
EX6061 50 Preps Store at 2-8°C

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

Kit content

Mini spin columns	**50x**
Collection tubes (1.5 ml)	**50x**
Lysis Buffer	**20 ml**
Precipitation Buffer	**15 ml**
Wash Buffer I	**20 ml**
Wash Buffer II	**40 ml**
Elution Buffer	**5 ml**

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. 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

Kit contains all ingredients for quick preparation of pure nucleic acids (NA) from biological samples like: serum, plasma, virus-infected cell culture supernatant.

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.

SinaPure™Viral 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 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 SinaPure™ Viral. 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

Add 100µl serum, plasma, body fluid or virus-infected cell culture supernatant to 1.5 ml micro centrifuge tube contains400 µl 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 avoiding any nuclease activity keep samples freeze until NA 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 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 flow-through.*
4. 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 50µl***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).

*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 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 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.
- 30-50 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

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.
	Sample was frozen and thawed several times.	- Keep samples freeze 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. - Warm Lysis before purification - Extend homogenization step
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**.

Kit Quality Control
All components of this Kit are successfully tested in the NA purification from co infected HIV, HCV and HBV sera followed by cDNA synthesis, PCR and Nested PCR.

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

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