SinaPure TM DNA

Kit for the isolation of DNA from Cell culture, Tissues, Gram negative Bacteria and CSF

Cat. No.:EX6011 50 Preparations

Store kit contents at: Room Temperature Store Ributinase at -20oC

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It can also be used for DNA isolation from blood, plasma and serum.

Mini spin columns 50×	
Collection tubes (1.5 ml)	50×
Prelysis Buffer	5 ml
Ributinase	1 ml
Lysis Buffer	20 ml
Precipitation Buffer	15 ml
Wash Buffer I	20 ml
Wash Buffer II	40 ml
Elution Buffer	2×1250µl

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 2 years. Ributinase is delivered as a solution and should be stored upon arrival at -20 °C. This guarantees performance for 2 years. For long storage and to avoid any probable contamination, keep Elution Buffer at 2-8oC.

Please take care that columns, once opened, should be used instantly. Close bottles immediately after use.

Kit Description

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

SinaPureTM DNA kit system is one of the latest nucleic acid purification technologies. This kit presents remarkable features of timesaving, easy, prompt and high yield DNA purification. Basis of the technology is the binding of DNA to matrices including silica membrane filters in presences of high salts concentration and reversibly elution in low salt condition like elution buffer or 10mM Tris-HCl. This kit supplied with Ributinase or innovative enzyme blend for simultaneously protein and RNA degradation therefore there is no need for further RNase In treatment. Obtained DNA is suitable for downstream applications including PCR.

Important notes: please read before starting

Warm Pre lysis and Lysis buffer by placing in 37oC for 15 min and finally softly shake. Heat a water bath or heater block to 55°C. You need a bench top micro centrifuge (12.100×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.

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 SinaPureTM DNA(Gram Negative Bacteria). Contact of Lysis and wash I buffer Solutions with acids or bleach solution, liberate toxic gas. When handling human samples, follow recommended procedures for biohazardous materials.

Sample Preparation

-Cell Culture

In 2ml tube collect 3-6×106 cells by centrifugation (5 min at 1000RPM), rinse the cell pellet by PBS. Repeat the centrifugation step and discard supernatant completely by pipetting. Add 100µl of Prelysis Buffer and20 µl of Ributinase to the tube, vortex it and then place at 55oC for 30 minutes or more. Follow the laboratory protocol*. For the efficient DNA separation from cell components is not recommended to use more cells than 6×106. Usage of thawed cells is not recommended.

-Tissues

Cut 25-50 mg (for RNA active tissue 10mg) tissues to small pieces by scalpel or homogenizer. Alternately, it can grind by mortar and pestle in liquid nitrogen. Add 100µl of Pre lysis Buffer to a 1.5ml microcentrifuge tube and then add 20 µl of Ributinase, place at 55oC for 1 to 3 hr (up to complete digestion) Follow the laboratory protocol*. Usage of thawed samples is not recommended.

-**Gram negative bacterial cultures**

In 2 ml tube, Collect 2×109 bacterial cells (10-20mg) by centrifugation 5 min at 4000RPM. Add 100µl of Pre lysis Buffer and 20 µl of Ributinase to the tube, vortex it and then place at 55oC for 30 minutes or more. Follow the laboratory protocol*.Usage of thawed cells is not recommended.

-CSF

Centrifuge 1- 1.5 ml of CSF specimen at 10.000 RPM for 5 min. Discard supernatant. Add 100 µl Pre lysis Buffer and 5 µl of Ributinase, vortex and then place at 60oC water bath or heater block for 20 min. and follow the protocol.

Above procedure may use for urine sediment.

-Blood

Whole blood must be collected in EDTA(1mg/ml)- to prevent clotting and DNA degradation. DNA extracted from heparinized blood cannot be used for PCR.Typically 100µl of fresh blood is used for DNA isolation with the yield of ≠2.0µg. If the blood is to be stored for later use it can be left at 2-8°C for (no longer than) 2 weeks.

-**Sera**

Add 100 µl of serum or plasma in 1.5 ml micro centrifuge tube, and then follow the laboratory protocol. Usage of fresh sera samples is recommended.

For long-term storage the samples should be aliquoted in 100µl portions and kept at -20°C. To avoiding any nuclease activity keep samples freeze until DNA extraction. Therefore add warm lysis buffer to freeze blood samples and softly shake to complete defreezing and follow the protocol.

***Vortexing during incubation time may increase the DNA yield (every 5 minutes for 5 sec).**

Optional: If required, incubate at 95°C for 15 min to inactivate pathogens. Note that incubation at 95oC can lead to some DNA degradation

Protocol

Approximate time for total nucleic acid preparation≠15 min.

1. Add 100 µl of sample to a sterile 1.5 or 2 ml polypropylene tube (not included).
2. Add 400 µl Lysis buffer and vortex at max speed for 20 seconds.
3. Add 300 µl Precipitation solution and vortex at max speed for 5 seconds.
4. Transfer the solution to a spin column with collection tube (included) by pipetting.
5. Centrifuge the tube at (12.100×g, 13.000 rpm) for 1 min. Discard flow-through*.
6. Add 400 µl Wash buffer I to spin column. Centrifuge at 12.100×g (13.000 rpm) for 1 min. Discard flow-through.
7. Wash the spin column with 400 µl of Wash buffer II centrifugation at 12.100×g (13.000 rpm) for 1 min. Discard flow-through.
8. Wash the spin column with 400 µl of Wash buffer II by centrifugation at 12.100×g (13.000 rpm) for 1 min. Discard flow-through.
9. Centrifuge at 12.100×g (13.000 rpm) for 1 min.**
10. Carefully transfer the column to a new 2 ml tube(not included). Place 50µl 65°C pre heated elution buffer in the center of the column, close lid and incubate for 3-5 min at 65oC. Thereafter, centrifuge at 12.100×g (13.000 rpm) for 1 min to elute the DNA.***

*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 DNA amounts are expected, a higher elution volume may increase the DNA yield. Generally, 30-50 µl elution volume gives satisfactory results. An alternative way of increasing the DNA yield is repeated centrifugation. Transfer 25-45µl eluted DNA to center of membrane filter again and centrifuge at 12.100×g (13.000 rpm) for 1 min to increase the DNA yield.

Troubleshooting

This guide may help solve problems that may arise.

Observation	Possible cause	Comments/suggestions
Low or no DNA yield	Inefficient lysis of sample	Make sure that: 1-incubation time with Ributinase 2-homogenization step by vortex were enough. - Decrease starting materials, extend incubation time and homogenization step.
-	Sample was frozen and thawed several times.	Keep samples freezed until DNA extraction. Whenever possible, fresh samples should be used and processed immediately. Storage at -20 °C is possible for several months. Several freeze-thaw cycles should be avoided, because this can result decreased molecular size of the DNA. - 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 enzymatic incubation time, homogenization step or remove particles.
DNA "smear"	Nuclease activity	Upon disintegration of Samples, cellular nucleases are released and may degrade genomic DNA. - Use only sterilized glass and plastic ware in order to avoid nuclease Contamination. - Make sure that you followed all washing steps of the procedure. Eventually repeat washing steps (I and II) once more.
Low DNA 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×g (13.000 rpm) for 1 min more**

DNA quality control

Agarose gel electrophoresis of prepared DNA is a direct method for testing DNA quality in terms of molecular size and conformation. Depending on the expected amount, pipette 5-10µleluted DNA directly to a gel slot. As for whole blood, DNA yield depends on quantity of leukocyte cells and storage duration and condition of sample.

Since DNA quantity is too small, Viral DNA from sera samples is invisible in agarose gel and not detectable spectrophotometery.

Photometric determination of DNA concentration and quality:

Determination of DNA concentration is done by UV reading at 260 nm. 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 = A260nm /A280nm. For a pure DNA preparation, Q lies between 1.7 and 2.0.

Kit Quality Control

All components of this Kit are successfully tested in the DNA purification and amplification reaction for:

Freeze or fresh whole blood for Thalassemia gene, urease positive gastric biopsies for H.pylori and House keeping gene detection and finally 100 µl of positive serum for HBV-qPCR.

For Research Use Only

SinaClonBioScience

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