

SinaPureTM DNA

Kit for the isolation of DNA from Whole blood, Serum & Plasma.

For Research Use Only

Cat. No.: EX6001

20 Preparations

Store kit contents at Room Temperature

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SinaClonBioScience

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SinaPure™ DNA

EX6001

50 Preps

Store at RT

Kit for the isolation of DNA from Whole blood, Serum & Plasma.

| | |
|---------------------------|--------|
| Mini spin columns | 20x |
| Collection tubes (1.5 ml) | 20x |
| Lysis Buffer | 8 ml |
| Precipitation Buffer | 6 ml |
| Wash Buffer I | 8 ml |
| Wash Buffer II | 16 ml |
| Elution Buffer | 1.5 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 2 years. Opened bags should be closed properly and stored in the refrigerator (2-8 °C). This guarantees performance for 2 years.

Kit Description

This kit contains all ingredients for quick preparation of pure DNA from blood, serum or plasma. 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.

SinaPure™ 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. Obtained DNA is suitable for downstream applications including PCR.

Sample Preparation

-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 2-4°C for (no longer than) 2 weeks. 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.

Important notes: please read before starting

Pre-warm Lysis buffer by placing in 37°C for 15 min and finally softly shake. Heat a water bath or heater block to 65°C. You need a bench top micro centrifuge (12.100xg, 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 SinaPure™ 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.

All steps perform at Room temperature.

Therefore add warm lysis buffer to freeze blood samples and softly shake to complete defreezing and follow the protocol.

-Sera

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

If it is impossible, samples should be frozen in aliquots of 100ul and stored at -20°C.

Protocol

Approximate time for total nucleic acid preparation from blood#15 min.

1. Add 100 μ l of sample to a sterile 1.5 ml polypropylene tube (not included).
2. Add 400 μ l Lysis buffer and vortex at max speed for 20 seconds.
3. Add 300 μ l 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 x g, 13.000 rpm) for 1 min. Discard flow-through.*
6. Add 400 μ l Wash buffer I to the spin column. Centrifuge at 12.100 x g (13.000 rpm) for 1 min. Discard flow-through.
7. Wash the spin column with 400 μ l of Wash buffer II by centrifugation at 12.100 x g (13.000 rpm) for 1 min. Discard flow-through.
8. Wash the spin column with 400 μ l of Wash buffer II by centrifugation at 12.100 x g (13.000 rpm) for 1 min. Discard flow-through.
9. Repeat the centrifugation step.**
10. Carefully transfer the column to a new 2 ml tube(Not included). Place 30 μ l 65°C pre heated elution buffer in the center of the column, close lid and incubate for 3-5 min at 65°C. Thereafter, centrifuge at 12.100 xg (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 μ l elution volume gives satisfactory results. An alternative way of increasing the DNA yield is repeated centrifugation. Transfer 25 μ l eluted DNA to a membrane filter again and centrifuge at 12.100xg (13.000 rpm) for 1 min to increase the DNA yield.

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Troubleshooting(for blood)

This guide may help solve problems that may arise.

| Observation | Possible cause | Comments/suggestions |
|----------------------------|--|--|
| Low or no DNA yield | Inefficient lysis of sample Sample was frozen and thawed several times. Sample was stored at 2-4°C longer than 2 weeks. Blood clots were present in the sample. | Make sure that homogenization step by vortex was enough. Take new sample. |
| | Nuclease activity/ contamination, Salt in elute | Upon disintegration of Samples, cellular nucleases are released and may degrade genomic DNA. Keep samples frozen 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. 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) |

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 5-10ul eluted 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 by spectrophotometry.

- 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:

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

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, and 100 µl of positive serum for HBV-PCR.

With this product, you may also need to other SinaClon products:

| | |
|--------|-------------------------------|
| MM2121 | Loading Buffer |
| EP5052 | Agarose |
| EP5041 | 5X TBE electrophoresis buffer |
| CH8161 | Water nuclease free |

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