SinaPure™ DNA

Kit for the isolation of DNA from Whole Blood

EX6001: 50 Preps

Store kit contents at: RT

Keep enzyme at: -20°C

Keep tubes and bottles tightly closed.

Kit Contents	Quantity/ Volume	Unit	Storage Condition
Spin columns	50	PCS	RT
Collection tubes	150	PCS	RT
Proteinase K	1	ml	***-20***
Lysis Buffer	10	ml	RT
Precipitation Buffer	10	ml	RT
Wash I Buffer	30	ml	RT
Wash II Buffer	50	ml	RT
Elution Buffer	5	ml	RT

Storage and Stability

The kit performance guaranteed until the printed expiration date. When the contents keep in the appropriate condition.

Kit Description

This kit contains the most necessary reagents for quick and Pure DNA preparation by spin column method from the recommended starting material:

Whole Blood:

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 released DNA to matrices including silica membrane filters in presences of high salts concentration and reversibly elution in low salt condition. Obtained DNA is suitable for downstream applications including PCR.

Principle:

Blood samples would lysed and degraded during incubation at the appropriate temperatures and in presence of Lysis buffer. Meanwhile, proteinase K accelerates the lysis reaction. This special enzyme would eliminate or reduce unwanted proteins contents of the cells simultaneously.

After successive washing steps, pure DNA eluted in low salt conditions.

Required Materials and equipment that not provided:

- Heater block or water bath (56°C)
- Bench top micro centrifuge (12.000 RPM)
- Precision pipettes and sterile pipette tips (10 to 100 and up to 1000µl).
- Sterile 1.5 ml or 2 ml polypropylene tubes

Important notes: please read before starting

- Follow GLP rules when performing nucleic acid extraction from biological samples.
- Check the procedure with a known sample, if use the kit for the first time.
- Check the vials for any possible crystals formation. In this case, place the vials at 37°C for 15 minutes and softly shake before use.
- Avoid freezing and thawing the samples.
- Use fresh samples.
- Keep the remaining initial samples preferably in small portions (200µl) or recommended volumes at minus -20 or colder.
- Aliquoting the enzymes may prolong the efficacy.
- Open spin columns only directly before use.

WARNINGS AND PRECAUTIONS

Caution: Avoid contact any kit reagents with skin & eyes. Wear gloves before use any extraction kit. When handling clinical samples, follow recommended procedures for biohazardous materials.

All steps should performed at Room temperature.

Sample Preparation

Whole blood should collected in EDTA (1mg/ml) to prevent blood clotting. Heparinized blood not recommended.

Typically, 200µl of fresh blood used for DNA isolation.

The blood can be kept at 2-8°C for (no longer than) 2 weeks for later use.

For long-term storage, aliquot samples into 200µl portions and keep at -20°C. To avoid any nuclease activity, keep samples frozen until DNA extraction.

Short Protocol:

Protocol

Lysis step:

1	Whole Blood	200µl
2	Proteinase K	20µl
3	Lysis Buffer	200µl

Mix the tube immediately and vortex for 20 seconds.

Incubate the tube for 18-30 minutes at 56°C; It may extend more.

Precipitation step:

4	Precipitation Buffer	200µl

Invert the tube and vortex for 3-5 seconds.

5	Transfer the tube content to the spin column.
6	Centrifuge the tube at (12,000 rpm) for 1 min and discard the flow-through.
7	Place the spin column in a new collection tube (provided).
8	Add 300µl Wash I buffer to spin column. Centrifuge the tube at (12,000 rpm) for 1 min. Discard the flow-through.
9	Add 300µl Wash I buffer to spin column. Centrifuge the tube at (12,000 rpm) for 1 min.
10	Place the spin column in a new collection tube (provided).
11	Add 700µl Wash buffer II to spin column. Centrifuge the tube at (12,000 rpm) for 1 min.
12	Discard the flow-through.
13	Centrifuge the tube at (12,000 rpm) for 3 minutes. Open the lid and incubate at 56°C for about 3-5 minutes.
14	Carefully transfer the column to a new 1.5ml tube (not included). Place 70 µl preheated elution buffer in the center of the column, close the lid and incubate at room temperature for about 5-10 minutes.
15	Centrifuge the tube at (12,000 rpm) for 1 min to elute the DNA.

The final yield increased if:

Repeat centrifugation. Transfer eluted DNA to the center of the membrane once again and after a short incubation at room temperature (about 2 minutes) centrifuge at 12,000 RPM for 1 min.

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 56˚C for 3-5 min.

Tips and Suggestion

Final elution volume depends on the initial sample: If higher DNA amounts needed, a higher elution volume may use. Accordingly, a higher concentration may achieved by a lower amount of elution buffer.
Generally, 50-100µl elution volume gives satisfactory results. An alternative way of increasing the DNA yield is repeated centrifugation. Transfer eluted DNA to the membrane filter once again and after a short incubation at room temperature (about 2 minutes) centrifuge at 12,000 RPM for 1 min.

Troubleshooting

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 2-8°C longer than 2 weeks. Blood clots were present in the sample.	Make sure that homogenization step by vortex was enough. Take new sample.
DNA "smear" **Low DNA performance**	Nuclease activity/ contamination, Salt in elute	Upon disintegration of Samples, cellular nucleases may release and the genomic DNA may get degraded. Keep the samples frozen until the DNA extraction. Whenever possible, use fresh sample and process it immediately. Storage at -20 °C is possible for several months. Multiple freeze-thaw cycles should avoided, because this can result in decreased the DNA molecular size. 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

1- 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.

2- Photometric determination of DNA concentration and quality:
Determination of DNA concentration is by UV reading at 260 nm. DNA preparations should be vortexed shortly and diluted (if needed) accordingly by 10mM 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 successfully tested for DNA purification and amplification reaction of:
Frozen or fresh whole blood.

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