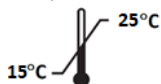


**SinaPure™ DNA**

(Kit for the isolation of DNA from Whole Blood)

**REF** EX6001

**Σ** 50 TESTS



**RUO**

Keep enzyme at -20°C

**Components**

Kit Contents	Quantity/ Volume	Storage Condition
Spin columns	50PCS	RT
Additional Collection tubes	50PCS	RT
Proteinase K	1.25ml	<b>-20°C</b>
Lysis Buffer	10ml	RT
Precipitation Buffer	10ml	RT
Wash I Buffer	32.5ml	RT
Wash II Buffer	30ml	RT
Elution Buffer	5ml	RT

**Description**

This kit contains the most necessary reagents for quick and Pure DNA preparation by spin column method from the **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 lyse 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.
- After successive washing steps, pure DNA eluted in low salt conditions.

**Required Materials and equipment that not provided**

- Heater block or water bath (59 °C)
- Bench top micro centrifuge (12,000rpm)
- Precision pipettes and sterile pipette tips (10 to 100 and up to 1000µl).
- Sterile 1.5ml or 2ml polypropylene tubes

**Warnings and Precautions**

Follow GLP rules when performing nucleic acid extraction from biological samples. Avoid contact any kit reagents with skin & eyes. Wear PPE1 before use any extraction kit. When handling biological samples, follow recommended procedures for biohazardous materials.

**Important notes: please read before starting**

1. Check the procedure with a known sample, if use the kit for the first time.
2. Check the vials for any possible crystal formation. In this case, place the vials at 37°C for 15 minutes and softly shake before use.
3. Avoid freezing and thawing the enzymes.
4. Aliquoting the enzymes may prolong their efficacy.
5. Use fresh samples.
6. Whole blood should be collected in EDTA (1mg/ml) to prevent blood clotting. Heparinized blood is not recommended.
7. The blood can be kept at 2-8°C for (no longer than) 2 weeks for later use. For long-term storage, samples preferably in small portions or recommended volumes at -20°C or colder (Typically, 200µl of fresh blood is used for DNA isolation). To avoid any nuclease activity, keep samples frozen until DNA extraction.
8. Open spin columns only directly before use.
9. All steps should be performed at Room temperature.

**Protocol**

1. Add 25µl Proteinase K to the empty tube.
2. Add 200µl fresh blood to the tube.
3. Add 200µl Lysis buffer to the tube.
4. Vortex vigorously for 20 seconds.
5. Incubate at 59 °C for 30 minutes.
6. let it cool down at RT for 2 minutes.
7. Add 200µl Precipitation buffer. Invert 3-5 times and transfer all tube content to the column quickly.
8. Centrifuge at 12,000rpm for 1min and discard the collection tube. Then put the column in the new collection tube (provided).
9. Add 650µl Wash I buffer, Centrifuge at 12,000rpm for 1min and discard the filtrate.

10. Add 600µl wash II buffer, Centrifuge at 12,000rpm for 1min and discard the filtrate.

11. To remove the excess amount of wash II buffer, centrifuge the empty column at 12,000rpm for 3min and discard the collection tube. Check the tubes for any droplet remaining. In this case, place the tubes with the open lid into the heater block for 3-5min to evaporate any remaining ethanol.

12. Carefully transfer the column to a new 1.5ml tube (not included). Place 70-100µl preheated elution buffer in the center of the column, close the lid and incubate at room temperature for 5-7min\*.

\* Final elution volume depends on the initial sample: If higher DNA amounts needed, a higher elution volume may use. Accordingly, a higher concentration may achieve by a lower amount of elution buffer.

13. Centrifuge the tube at 12,000rpm for 1min to elute the DNA.

The final yield increased if:

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

- Extend the incubation time in step 11 to about 10 min. (with closed lids).

- Reduce elution volume to 50µl. (This can increase DNA concentration but reduce the final yield).

### Quality Control

#### 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 3-5µl eluted DNA directly to a 1% gel slot. DNA yield depends on quantity and quality of cells and storage duration and condition of sample.

#### - Photometric determination of DNA concentration and quality:

Determination of DNA concentration is done by UV reading at 260nm. 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:  $Q = A_{260nm} / A_{280nm}$  (For a pure DNA preparation, Q lies between 1.7 and 2.0)

Evaluate and prove amplifiable quality of extracted DNA by amplification of a housekeeping gene. Inhibitors may remove by further purification.

### Kit Quality Control






All components of this Kit are successfully tested in the DNA purification and amplification reaction for freeze and fresh whole blood.

### Troubleshooting


This guide may help solve problems that may arise.

Observation	Possible cause	Comments/suggestions
<b>Low or no DNA yield</b>	Inefficient lysis of sample	Make sure that homogenization step by vortex was enough.
	Blood clots were present in the sample.	Take new sample.
	Sample was frozen and thawed several times.	
	Sample was stored at 2-8°C longer than 2 weeks.	
<b>DNA "smear" and Low DNA performance</b>	Nuclease release and activity/ contamination, Salt in elute	<ul style="list-style-type: none"> <li>- Keep the samples frozen until the DNA extraction.</li> <li>- Use fresh sample and process it immediately.</li> <li>- Multiple freeze-thaw cycles should be avoided.</li> <li>- Use only sterilized glass and plastic ware in order to avoid nuclease Contamination.</li> <li>- Repeat washing steps (I and II)</li> </ul>

### Signs

Signs	Definitions	Signs	Definitions
	Temperature range on product use		Name and address of the manufacturer of the product
	For Research Use Only		
	Product shipping conditions		

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