SinaPure™ DNA

(Kit for the isolation of DNA from Cell Culture, Tissue, Whole Blood & Gram-Negative Bacteria)

REF EX6011 | Σ 50 TESTS | RUO | 15°C – 25°C

Keep enzyme at -20°C

Components

Kit Contents	Quantity/ Volume	Storage Condition
Spin columns	50PCS	RT
Additional collection tubes	50PCS	RT
PreLysis solution	10ml	RT
Proteinase K	1.25ml	**-20°C**
Lysis solution	10ml	RT
Precipitation solution	10ml	RT
Wash I solution	60ml	RT
Wash II solution	60ml	RT
Elution Buffer	5ml	RT

Description

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.

This kit contains the most necessary and ready to use reagents for quick and Pure DNA preparation by spin column method from the recommended starting material: **Cell culture, Tissue, Whole Blood and Gram-Negative Bacteria**

Principle

Samples would be degraded during incubation at the appropriate temperatures and in presence of PreLysis and Lysis solution. Meanwhile, Proteinase K accelerates the reaction by eliminating or reducing unwanted proteins of the cells. After successive washing steps, pure DNA would be released in low salt conditions.

Required Materials and equipment that were not provided

1. Heater block or water bath
2. Bench top micro centrifuge (12000rpm)
3. Precision pipettes and sterile pipette tips (10 to 100 and up to 1000µl)
4. Sterile 1.5ml or 2ml polypropylene tubes

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 enzyme.
4. Use fresh samples.
5. Keep the remaining initial samples preferably in small portions (200µl, 20mg…) or recommended volumes at -20˚C or colder.
6. Aliquoting the enzyme may prolong their efficacy.
7. Open spin columns only directly before use.
8. All steps should be performed at Room Temperature.
9. When RNA-free DNA is required, Ribonuclease A (Cat. No.: MO5411) should be added (it is not provided).

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.

Samples Preparation

- Cell Culture

1. Collect cells (1-3x10^6) by centrifugation at 5000rpm for 5 minutes.
2. Dislodge the cell pellet by soft shaking or by finger tapping until it forms a complete suspension.
3. Add 200µl PreLysis solution to the cell suspension.
4. Add 5µl Ribonuclease A. Vortex vigorously for 10 seconds and incubate at 37℃ for 15 minutes **(Optional)**.
5. Add 25µl Proteinase K.
6. Vortex vigorously for 10 seconds.
7. Incubate at 59°C for 30 minutes. Occasionally vortex (every 5-10min). Short vortex during incubation may enhance the final yield.
8. Add 200µl Lysis solution to the tube and vortex vigorously for 10 seconds.
9. Incubate the tube at 59°C for 10 minutes. In case of any remaining particles, centrifuge the tube briefly and transfer the upper phase to the new tube.
10. let it cool down at RT for 2 minutes.

Follow the laboratory protocol 1.

- Gram Negative Bacteria

1. Collect bacterial cells (up to 2x10^9) by centrifugation at 5000rpm for 5 minutes.
2. Dislodge the cell pellet by soft shaking or by finger tapping until it forms a complete suspension.
3. Add 200µl PreLysis solution to the cell suspension.
4. Add 5µl Ribonuclease A. Vortex vigorously for 10 seconds and incubate at 37℃ for 15 minutes **(Optional)**.
5. Add 25µl Proteinase K.
6. Vortex vigorously for 10 seconds.
7. Incubate at 59°C for 30 minutes. Occasionally vortex (every 5-10min). Short vortex during incubation may enhance the final yield.
8. Add 200µl Lysis solution to the tube and vortex vigorously for 10 seconds.
9. Incubate the tube at 59°C for 10 minutes. In case of any remaining particles, centrifuge the tube briefly and transfer the upper phase to the new tube.
10. let it cool down at RT for 2 minutes.

Follow the laboratory protocol 1.

- Blood

For the isolation of DNA from Whole blood, SinaPure™ Blood DNA (Cat. No. EX6001) is recommended. Whole blood should be collected in EDTA (1mg/ml) to prevent blood clotting. Heparinized blood is not recommended.
Typically, 200µl of fresh blood is 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, samples should be aliquoted in 200µl portions and kept at -20°C. To avoid any nuclease activity, keep samples frozen until DNA extraction.

1. Add 200µl Lysis solution to the empty tube.
2. Add 25µl Proteinase K
3. Add 5µl Ribonuclease A **(Optional)**.
4. Add 200µl fresh blood to the tube.
5. Vortex vigorously for 20 seconds.
6. Incubate at 70°C for 10 minutes.
7. let it cool down at RT for 2 minutes.

Follow the laboratory protocol 2.

- Tissue

Cut 5-20mg (maximum) of selected tissue and cut it into small pieces with a scalpel or homogenizer. Alternately, it can grind by mortar and pestle in liquid nitrogen. Freeze and thawed samples are not recommended.
Final yields of DNA may vary depends on the different tissue types. In general, we found satisfactory results from 5mg to maximum 20mg of mice tissues. However, in some cases you may need to optimize the initial starting materials due to different levels of nucleic acids in specific tissue. In addition, the quality of material will affect DNA yield.

1. Add 200µl PreLysis solution to the sample.
2. Add 25µl Proteinase K.
3. Add 5µl Ribonuclease A **(Optional).**
4. Vortex vigorously for 10 seconds.
5. Incubate at 59°C for 1 to 3 hours (up to complete digestion). Occasionally vortex (every 5-10min). Short vortex during incubation may enhance the final yield.
   - For some tissues, incubation may continue overnight. In this case, complete digestion may need additional Proteinase K (20µl- Not provided).
6. Add 200µl Lysis solution to the tube and vortex vigorously for 10 seconds.
7. Incubate the tube at 59°C for 10 minutes. In case of any remaining particles, centrifuge the tube briefly and transfer the upper phase to a new tube.
8. let it cool down at RT for 2 minutes.

Follow the laboratory protocol 1.

laboratory protocol 1

1. Add 200µl Precipitation solution. Invert 3-5 times and transfer all tube content to the column quickly.
2. Centrifuge at 12,000rpm for 1min and discard the collection tube. Then put the column in the new collection tube (provided).
3. Add 600µl Wash I solution, Centrifuge at 12,000rpm for 1min and discard the filtrate.
4. Repeat step 3.
5. Add 600µl wash II solution, Centrifuge at 12,000rpm for 1min and discard the filtrate.
6. Repeat step 5.
7. To remove the excess amount of wash II solution, centrifuge the empty column at 12,000rpm for 3min and discard the collection tube.
8. 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*.
9. Centrifuge the tube at 12,000rpm for 1min to elute the DNA.
10. Transfer eluted DNA to the center of the membrane once again and centrifuge at 12,000rpm for 1min.

laboratory protocol 2

1. Add 200µl Precipitation buffer. Invert 3-5 times and transfer all tube content to the column quickly.
2. Centrifuge at 12,000rpm for 1min and discard the collection tube. Then put the column in the new collection tube (provided).
3. Add 650µl Wash I solution, Centrifuge at 12,000rpm for 1min and discard the filtrate.
4. Add 600µl wash II solution, Centrifuge at 12,000rpm for 1min and discard the filtrate.
5. To remove the excess amount of wash II solution, centrifuge the empty column at 12,000rpm for 3min and discard the collection tube.
6. 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*.
7. Centrifuge the tube at 12,000rpm for 1min to elute the DNA.
8. Transfer eluted DNA to the center of the membrane once again and centrifuge at 12,000rpm for 1min.

* Elution volume depends on the initial sample. If higher DNA amounts are needed, a higher elution volume may use. Accordingly, a higher concentration may be achieved by a lower amount of Elution buffer.

Quality Control

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

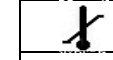
2. Photometric determination of DNA concentration and quality:
Determination of DNA concentration is done by UV reading at 260nm. DNA preparations should be vortexed shortly and diluted (if needed) accordingly by 10mM Tris-HCl or Elution buffer. Blank and dilution buffer should be the same. A standard procedure of measuring DNA quality is the determination of the absorption quotient (Q) of readings at A260nm and A280nm:
Q = A260nm /A280nm
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 Tissue, cell culture, gram negative bacteria and freeze or fresh whole blood.

Signs

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

Troubleshooting

This guide may help solve problems that may arise.

Observation	Possible cause	Comments/suggestions
Low or no DNA yield	Inefficient lysis of sample	- Keep the samples at suitable storage temperatures (fridge or frozen) in pre-weighted and aliquoted portions. For frozen and aliquoted samples (cell and bacterial pellets, tissues, blood...), add warm PreLysis or Lysis solutions before de-freezing. - Make sure that the collected cells (bacteria and cultured cells) were dislodged completely by finger blows and formed a homogenous suspension by several inverting, softly shaking of the tubes, or by the other appropriate manners like frequent pipetting. - Depending on the cell cycles, quantities, and nucleic acid contents it may need to optimize the initial amounts of samples including bacteria, cultured cells, whole blood volume, or tissue sizes and weights. In those cases, it would be better to optimize the protocol with a known sample. - You may need to homogenize the initial sample with the appropriate protocol and increase incubation time with the proteinase K enzyme or decrease the starting materials.
	Sample was frozen and thawed several times.	- Keep samples freeze until DNA extraction. 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 be clogged during purification	- Absolutely avoid transferring any remaining particles, tissues, undigested or undissolved materials after the recommended incubation time and temperature with Lysis buffer. Briefly centrifuge the content and transfer the upper homogenous liquid to a new tube. - Extend enzymatic incubation time, homogenization step or remove particles. - Reduce the amount of initial sample.

Observation	Possible cause	Comments/suggestions
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.
Increased absorbance at 230 or 280 nm wavelengths	-	- In general, and after successive washing steps the absorbances remain at the acceptable ranges and would not affect downstream applications, especially the activity of the Taq DNA Polymerase. Otherwise, you may need to extract the eluted DNA from the first step once again or use a new sample.
		- Ensure that the column is dry and no ethanol contaminates is at the tip of the column. If you observe residues of ethanol. Repeat the step 7 again in laboratory protocol 1 and step 5 again in laboratory protocol 2.

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