SinaPure™ DNA

(Kit for the isolation of DNA from Gram Positive Bacteria)

EX6021

50 TESTS

RUO

(Keep enzymes at: -20°C)

Components

Kit Contents	Quantity/ Volume	Storage Condition
Spin columns & Collection tubes	50PCS	RT
Lysozyme	50mg	-20
Ribonuclease A	250µl	-20
Proteinase K	1250µl	-20
Lysozyme Solution	5ml	RT
PreLysis Solution	5ml	RT
Lysis Solution	10ml	RT
Precipitation Solution	15ml	RT
Wash I Solution	50ml	RT
Wash II Solution	60ml	RT
Elution Buffer	5ml	RT

Description

SinaPure™ DNA kit system is one of the latest nucleic acid purification technologies that presents remarkable features of timesaving, easy, prompt and high yield DNA purification. This kit contains the most necessary reagents for quick and Pure DNA preparation by spin column method from **Gram-Positive Bacteria and** 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

Cell wall of Gram-Positive Bacteria would be degraded during incubation at the appropriate temperatures and in presence of Lysozyme. Meanwhile, Proteinase K and Ribonuclease A enzymes will reduce unwanted proteins and RNA contents of the cells simultaneously. After successive washing steps, pure DNA would be released in low salt conditions.

Warnings and Precautions

1. Follow GLP rules when performing nucleic acid extraction from biological samples.
2. Avoid contact of any kit reagents with skin & eyes and Wear PPE_1 before use any extraction kit.
3. Check the procedure with a known sample, if use the kit for the first time.
4. Use fresh samples.
5. All steps should be performed at Room temperature.
6. Check the vials for any possible crystal formation. In this case, place the vials at 37°C for 15minutes and softly shake before use.
7. Avoid freezing and thawing the samples.
8. Aliquoting the enzymes may prolong their efficacy.
9. Open spin columns only directly before use.

Required materials and tools that are not supplied with the kit:

Row	Materials and Tools
1	Heater block or water bath (37-58˚C)
2	Bench top micro centrifuge (12.000rpm)
3	Precision pipettes and sterile pipette tips (10 to 100 and up to 1000µl)
4	Sterile 1.5ml or 2ml polypropylene tubes

Protocol

A. Lysozyme Preparation

1. Add 5ml Lysozyme Solution to Lysozyme enzyme powder (10mg/ml) and pipette until enzyme is well dissolved. Then aliquot the solution into suitable volumes and store at -20˚C and avoid freezing and thawing.

B. Samples Preparation

2. Collect gram positive bacterial cultures (5ml, OD=1) by centrifugation for 5min at 4500rpm.
3. Resuspend bacterial pellet in 100µl Lysozyme solution plus 100µl PreLysis solution. Vortex vigorously for 10 seconds and incubate at 37˚C for at least 1-2 hours. For some samples, may be required to overnight incubation.
4. Increase temperature to 58˚C, add 200µl Lysis solution plus 25µl Proteinase K. Mix and incubate at 58˚C for 30-40min [1*].

Optional: After step 4, If required, incubate at 95°C for 15min to inactivate pathogens [2*].

5. Centrifuge the tube at 8,000rpm for 3min and transfer the upper phase to a new tube.
6. Reduce temperature to 37˚C and Add 5µl Ribonuclease A. Mix and incubate at 37˚C for 20min.

C. Extraction Protocol

7. Add 300µl Precipitation solution. Invert three times, incubate at room temperature for 3min and transfer tube content to the column.
8. Centrifuge at 12,000rpm for 1min and discard the filtrate.
9. Add 500µl Wash I solution, Centrifuge at 12,000rpm for 1min and discard the filtrate.
10. Again add 500µl Wash I solution, Centrifuge at 12,000rpm for 1min and discard the filtrate.
11. Add 600µl Wash II solution, Centrifuge at 12,000rpm for 1min and discard the filtrate.
12. Again add 600µl Wash II solution, Centrifuge at 12,000rpm for 1min and discard the filtrate.
13. To remove the excess amount of wash II solution, centrifuge the empty column for more 3min at 12,000rpm and discard the collection tube (3*).
14. Carefully transfer the column to a new 1.5ml tube (not included). Place 35µl preheated elution buffer in the center of the column, close the lid and incubate at room temperature for 5-7min.
15. Centrifuge the tube at (12,000rpm) for 1min to elute the DNA (4*).

Final elution volume depends on the initial sample. If higher DNA amounts are needed, a higher elution volume may be used. Accordingly, a higher concentration may be achieved by a lower amount of Elution buffer. Generally, 35-100µl elution volume gives satisfactory results.

Row	Starred notes
(1*)	Vortexing during incubation time of Proteinase K may increase the DNA yield (every 5min for 5 sec).
(2*)	It should be noted that it can lead to some DNA degradation.
(3*)	Avoid contaminating the column with ethanol. Ensure that the column is dry and no ethanol contaminates at the tip of the column. In case of observing any residues of ethanol, place the column in 58˚C for 3-5min.
(4*)	The final yield can be increased if - Repeat centrifugation. Transfer eluted DNA to the center of the membrane once again and after a short incubation at room temperature (about 2min) centrifuge at 12,000rpm for 1min.

Quality Control

A. 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 gel slot. For gram positive bacteria, 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 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.

B. Kit Quality Control:

All components of this Kit are successfully tested in the DNA purification and amplification reaction for gram positive bacteria.

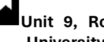
Signs

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

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 freeze-thawed several times.	- Make sure of enough vortexing during Proteinase K step. - Using fresh culture is recommended.
DNA "smear"	- Nuclease activity/ contamination	- Upon disintegration of samples, cellular nucleases are released and may degrade genomic DNA. Whenever possible, fresh samples should be used and processed immediately. Several freeze-thaw cycles should be avoided, because this can result in decreased molecular size of the DNA. - Use only sterilized glass and plastic ware in order to avoid nuclease Contamination.
Low DNA	- Salt in elute	- Make sure that you followed all washing steps of the procedure. Eventually repeat 70% ethanol washing.


Unit 9, Rouyesh building, Science and Technology Park, Tarbiat Modares University, Pajouhesh Blvd, Tehran, Iran

+98219108211 www.sinaclon.com hi@sinaclon.com