SinaPure™ DNA

Kit for the isolation of DNA from Gram Positive Bacteria

EX6021: 50 Preps

Store kit content at: RT

Keep enzymes at: -20°C

Keep tubes and bottles tightly closed.

Kit Contents	Quantity/ Volume	Unit	Storage Condition
Spin columns	50	PCS	RT
Collection tubes	50	PCS	RT
PreLysis solution	10	ml	RT
Lysozyme	1	ml	***-20***
Ributinase	1250	µl	***-20***
Lysis solution	10	ml	RT
Precipitation solution	10	ml	RT
Wash I solution	30	ml	RT
Wash II solution	50	ml	RT
Elution Buffer	5	ml	RT

Storage and Stability

The performance of the kit will be 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:

Gram Positive Bacteria

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:

Samples would be degraded during incubation at the appropriate temperatures and in presence of PreLysis and Lysis solution. Meanwhile, enzymes accelerate the reaction by eliminating or reducing unwanted proteins and RNA contents of the cells simultaneously.

After successive washing steps, pure DNA would be released in low salt conditions.

Required Materials and equipment that were not provided:

- Heater block or water bath (37-56°C)
- Bench top micro centrifuge (12.000 RPM)
- Precision pipettes and sterile pipette tips (10 to 100 and up to 1000 µl).
- 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 crystal 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.
- Aliquoting the enzymes may prolong their efficacy.
- Open spin columns only directly before use.

WARNINGS AND PRECAUTIONS

Caution: Avoid contact any kit reagents with skin & eyes. Wear PPE[1] before use any extraction kit. When handling biological samples, follow recommended procedures for biohazardous materials.

All steps should be performed at Room temperature.

Samples Preparation

-Gram positive bacterial cultures

1. Collect gram positive bacterial cultures (maximum $2x10^9$) by centrifugation for 10 min at 4500 rpm.
2. Resuspend bacterial pellet in 200µl G^+Prelysis solution vortex vigorously for 10 seconds and add 20µl Lysozyme. Mix and incubate at 37˚C for at least 30min (up to digest).
3. Increase temperature to 56˚C and add 25µl Ributinase. Mix and incubate for at least 30 min*
(Until complete cell lysis). Usage of thawed cells is not recommended.
Optional: If required, incubate at 95°C for 15 min to inactivate pathogens**

*** Vortexing during Ributinase incubation time may increase the DNA yield (every 5 minutes for 5 sec).**

**** Note that it can lead to some DNA degradation.**

4. Add 200 µl Lysis solution to the tube and vortex vigorously for 10 seconds.
5. Incubate the tube at 56˚C for 10 minutes.

Protocol:

1	Add 200 µl Precipitation solution. Invert three times and transfer all tube content to the column quickly.
2	Centrifuge at 12,000 RPM for 1 min and Discard the filtrate.
3	Add 300 µl Wash I solution.
4	Centrifuge at 12,000 RPM for 1 min and discard the filtrate.
5	Add 300 µl wash I solution.
6	Centrifuge at 12,000 RPM for 1 min and discard the filtrate.
7	Add 700 µl wash II solution, Centrifuge at 12,000 RPM for 1 min and discard the filtrate.
8	Centrifuge empty column at 12,000 RPM for **2** min and discard the filtrate.
9	Place the tubes with the open lid into the heater block for 3- 5 min to evaporate any remaining ethanol.
10	Add 70 µl Elution Buffer directly at the center of the membrane and store at room temperature for 2-3 min, Centrifuge at 12,000 RPM for 1 min.

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 2 minutes) centrifuge at 12,000 RPM for 1 min.
Extend the incubation time in step 10 to about 10 min. (with closed lids).

You can discard the collection tube and place the column in a new 1.5 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 56˚C for 3-5 min.

Tips and Suggestions

Final 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.
Generally, 50-100 µl 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.
For more purity you can add 500 µl wash II solution to the column in step 7 and centrifuge at 12,000 RPM for 1 min and repeate again. (provided)

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.	-Make sure that vortexing during Ributinase step was enough. -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 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.

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 3-5 ul eluted DNA directly to a gel slot. for gram negative 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 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 = A260nm /A280nm
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 gram positive bacteria