SinaPure™ DNA

Kit for the isolation of DNA from Gram Positive Bacteria

For Research Use Only

Cat. No.: EX6021 **50 Preparations**

Store kit contents at: RT **Store Enzymes at -20°C**

SinaClonBioScience

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EX6021 50 Preps Store at RT

Kit for the isolation of DNA from Gram Positive Bacteria

Mini spin columns	**50x**
Collection tubes (1.5 ml)	**50x**
G +Prelysis Buffer	**5 ml**
Lysozyme	**1ml**
Ributinase	**500ul**
Lysis Buffer	**20 ml**
Precipitation Buffer	**15 ml**
Wash Buffer I	**20 ml**
Wash Buffer II	**40 ml**
Elution Buffer	**2x1250ul**

Storage and Stability

Spin columns of the kit are packed in closed bags and show full performance in this state at room temperature (18-25°C) for at least 1 year. Lysozyme and Ributinase is delivered as a solution and should be store up on arrival at -20 °C. This guarantees performance for 1 year. For Lysozyme, it should be better to aliquots to several parts.
Please take care that columns, once opened, should be used instantly. Close bottles immediately after use.

Kit Description

This kit contains all ingredients for quick preparation of pure DNA from gram positive bacteria. The kit contains spin columns, enzymes, buffers and reagents necessary for lysis of material, DNA binding to the matrix, and washing and elution of DNA in to small volume from the matrix. Each kit contains a manual with detailed protocols of DNA extraction. 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 DNA to matrices including silica membrane filters in presences of high salts concentration and reversibly elution in low salt condition like elution buffer or 10mM Tris-HCl. This kit supplied with Lysozyme or muramidase. It catalyses the hydrolysis of N-acetylmuramide link ages in bacterial cell walls. Lysozyme breaks down the cell walls of bacteria and it used to prepare spheroplasts. Gram-positive cells are quite susceptible to this hydrolysis as their cell walls have a high proportion of peptidoglycan. Special ingredients of G +Prelysis Buffer also help for more efficient and quick cell wall degradation. This kit also has, Ributinase or innovative enzyme blend for simultaneously protein and RNA degradation therefore there is no need for further RNasin treatment. Obtained DNA is suitable for downstream applications including PCR.

Important notes: please read before starting

Pre-warm Lysis buffer by placing in 37°C for 15 min and finally softly shake. You need a bench top micro centrifuge (12.100 x g, 13.000 rpm), precision pipettes and sterile pipette tips allowing pipetting volumes 1 to 10 µl, up to 100 µl and up to 1000µl, and sterile 1.5 ml or 2 ml polypropylene tubes. Place spin columns in a rack before starting with the extraction protocol. Open spin columns only directly before use.

WARNINGS AND PRECAUTIONS

Caution: Lysis and wash I Buffer are toxic and irritant. They should be open in fume hood.
Avoid contact any kit reagents with skin & eyes. Wear gloves before use SinaPure™ DNA(Gram Positive Bacteria). Contact of Lysis and wash I buffer Solutions with acids or bleach solution, liberate toxic gas. When handling human samples, follow recommended procedures for biohazardous materials.

Sample Preparation

-Gram positive bacterial cultures

Collect gram positive bacterial cultures (10-20mg or maximum $2x10^9$)by centrifugation for10min at 4500rpm. Resuspend bacterial pellet in 100µl G+Prelysis buffer and add 20µl Lyzosyme. Mix and incubate at 37°C for at least 30min. Increase temperature to 55°C and add 10µl Ributinase. Mix and incubate for at least 30 min*

(Until complete cell lysis) and follow the laboratory protocol. 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.**

Protocol

Approximate time for total nucleic acid preparation≠15 min.

1. Add 400µl Lysis buffer and vortex at max speed for 20 seconds.

2. Add 300µl Precipitation solution and vortex at max speed for 5 seconds.

3. Transfer the solution to a spin column with collection tube (included) by pipetting.

4. Centrifuge the tube at (12.100 x g, 13.000 rpm) for 1 min. Discard flow-through.*

5. Add 400 µl Wash buffer I to the spin column. Centrifuge at 12.100 x g (13.000 rpm) for 1 min. Discard flow-through.

6. Wash the spin column with 400 µl of Wash buffer II by centrifugation at 12.100 x g (13.000 rpm) for 1 min. Discard flow-through.

7. Wash the spin column with 400 µl of Wash buffer II by centrifugation at 12.100 x g (13.000 rpm) for 1 min. Discard flow-through.

8. Place spin column in collection tube. Centrifuge at 12.100 x g (13.000 rpm) for 1 min.**

9. Carefully transfer the column to a new 2 ml tube(not included). Place 30 µl 65°C pre heated elution buffer in the center of the column, close lid and incubate for 3-5 min at 65°C. Thereafter, centrifuge at 12.100 xg (13.000 rpm) for 1 min to elute the DNA.***

***You can discard collection tube and place column in new 2 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 a new reaction tube.**

*****The elution volume depends on the sample: If high DNA amounts are expected, a higher elution volume may increase the DNA yield. Generally, 30-50µl elution volume gives satisfactory results. An alternative way of increasing the DNA yield is repeated centrifugation. Transfer 25µl eluted DNA to a membrane filter again and centrifuge at 12.100 xg (13.000 rpm) for 1 min to increase the DNA yield.**

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 repeat70% 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 5-10ul eluted DNA directly to a gel slot.

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.

Kit Quality control

All components of this Kit are successfully tested in the DNA purification and amplification reactions from gram positive bacterial cultures.

With this product, you may also need to other SinaClon products

MM2121	Loading Buffer
EP5052	Agarose
EP5041	5X TBE electrophoresis buffer
CH8161	Water nuclease free

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