CinnaPure DNA

Kit for the isolation of DNA from Gram Positive Bacteria

Cat. No. :PR881614 **50 Preparations**

Store kit contents at:2-8˚C **Store Enzymes at -20°C**

CinnaGen Molecular Biology and Diagnostic Sale Office
Tajbakhsh St. 24th Km Karaj Makhsous Rd., AryoGen Complex. Tehran, Iran
Tel: +9826 3610 6485-6
Fax: +9826 3610 6487
Customer Service: +98 912 135 0829
Place you order: order@sinaclon.com, www.sinaclon.com
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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	500µl
Lysis Buffer	20 ml
Precipitation Buffer	15 ml
Wash Buffer I	20 ml
Wash Buffer II	40 ml
lution Buffer	2x1250µl

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 2 years. Lysozyme and Ributinase is delivered as a solution and should be store upon arrival at -20 ˚C. This guarantees performance for 2 years. For Lysozyme, it should be better to aliquots to several parts. For long storage and to avoid any probable contamination, keep Elution Buffer at 2-8^{o}C.
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 into small volume from the matrix. Each kit contains a manual with detailed protocols of DNA extraction.
CinnaPure –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 linkages 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^{o}C for 15 min and finally softly shake. You need a bench top micro centrifuge (12.100xg, 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.
Caution: Lysis and wash I Buffer are irritants.

Sample Preparation

-Gram positive bacterial cultures

Collect gram positive bacterial cultures (10-20mg or maximum $2x10^9$) by centrifugation for 10min 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 10ul 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 400 µl Lysis buffer and vortex at max speed for 20 seconds.

2. Add 300 µl 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 collection tube.

5. Place spin column in new collection tube (not included) and 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 1.5 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 x g (13.000 rpm) for 1 min to elute the DNA.*

***Avoid transferring any remaining particles or unsolved materials into spin column.**
****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, 30-50 µl elution volume gives satisfactory results. An alternative way of increasing the DNA yield is repeated centrifugation. Transfer 25 ul eluted DNA to a membrane filter again and centrifuge at 12.100 ×(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 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 reactions from gram positive bacterial cultures.

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

MR7733	Loading Buffer
MR7730	Agarose
MR7725	5xTBE electrophoresis buffer
DW8505	DNase free water