CinnaPure DNA

Kit for the isolation of DNA from whole blood, Serum &Plasma.

Cat. No.: PR881612 **50 Preparations**

Store kit contents at: 2-8°C

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Mini spin columns	50x
Collection tubes (1.5 ml)	50x
Lysis Buffer	20 ml
Precipitation Buffer	15 ml
Wash Buffer I	20 ml
Wash Buffer II	40 ml
Elution 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. Opened bags should be closed properly and stored in the refrigerator (2-8 ˚C). This guarantees performance for 2 years.

Kit Description
This kit contains all ingredients for quick preparation of pure DNA from blood, serum or plasma. The kit contains spin columns, 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. 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. Heat a water bath or heater block to 65˚C. 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 Buffer is an irritant.

Sample Preparation

-Blood
Whole blood must be collected in EDTA (1mg/ml)- to prevent clotting and DNA degradation. DNA extracted from heparinized blood cannot be used for PCR. Typically 100µl of fresh blood is used for DNA isolation with the yield of ≠2.0 µg. If the blood is to be stored for later use it can be left at 2-4˚C for (no longer than) 2 weeks. *For long-term storage the samples should be aliquoted in 100µl portions and kept at -20°C.* To avoiding any nuclease activity keep samples freeze until DNA extraction. Therefore add warm lysis buffer to freeze blood samples and softly shake to complete defreezing and follow the protocol.

-Sera
Add 100 ul of serum or plasma in 1.5 ml micro centrifuge tube, and then follow the laboratory protocol. Usage of fresh sera samples is recommended.
If it is impossible, *samples should be frozen in aliquots of 100µl and stored at -20°C.*

Protocol

Approximate time for total nucleic acid preparation from blood ≠ 15 min.

1. **Add 100 µl of sample to a sterile 1.5 ml polypropylene tube (not included).**
2. **Add 400 µl Lysis buffer and vortex at max speed for 20 seconds.**
3. **Add 300 µl Precipitation solution and vortex at max speed for 5 seconds.**
4. **Transfer the solution to a spin column with collection tube (included) by pipetting.**
5. **Centrifuge the tube at (12.100 x g, 13.000 rpm) for 1 min. Discard collection tube.**
6. **Place spin column in new collection tube (included) and add 400 µl Wash buffer I to the**
7. **spin column. Centrifuge at 12.100 x g (13.000 rpm) for 1 min. Discard flow-through.**
8. **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.**
9. **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.**
10. **Repeat the centrifugation step.***
11. **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 65ºC. Thereafter, centrifuge at 12.100 x g (13.000 rpm) for 1 min to elute the DNA.****

* 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 µl eluted DNA to a membrane filter again and centrifuge at 12.100xg (13.000 rpm) for 1 min to increase the DNA yield.

Troubleshooting (for blood)

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. Sample was stored at 2-4°C longer than 2 weeks. Blood clots were present in the sample.	Make sure that homogenization step by vortex was enough. Take new sample.
DNA "smear" **Low DNA performance**	Nuclease activity/ contamination, Salt in elute	Upon disintegration of Samples, cellular nucleases are released and may degrade genomic DNA. Keep samples freezed until DNA extraction. Whenever possible, fresh samples should be used and processed immediately. 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. Use only sterilized glass and plastic ware in order to avoid nuclease Contamination. Make sure that you followed all washing steps of the procedure. Eventually repeat washing steps (I and II)

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. As for whole blood, DNA yield depends on quantity of leukocyte cells and storage duration and condition of sample.
Since DNA quantity is too small, Viral DNA from sera samples is invisible in agarose gel and not detectable by spectophotometery.

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:
Freeze or fresh whole blood for Thalassemia gene, and 100 µl of positive serum for HBV-PCR.

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

MR7733	Loading Buffer
MR7730	Agarose
MR7725	5xTBE electrophoresis buffer
DW8505	Water nuclease free