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Circulating Nucleic Acid Mini Kit

Product Number: DNK4701

Shipping and Storage

1. Inappropriate storage at low temperatures (4℃ or -20℃) can cause solution precipitation, affecting the effectiveness of use. Therefore, transportation and storage are carried out at room temperature (15℃ -25℃).
2. Proteinase K is stored in a ready to use glycerol Buffer and transported at room temperature. After receipt, store at room temperature not exceeding 20℃ for at least 6 months, at 4℃ for 12 months, and at -20℃ for 2 years.
3. To avoid volatilization, oxidation, and pH changes caused by prolonged exposure of reagents to the air, each solution should be covered tightly in a timely manner after use.

Components

Component	Storage	DNK4701 50 Preps
Buffer ACL	RT	40 ml
Buffer ACB	RT	60 ml
Buffer PE	RT	16 ml
Buffer RW	RT	10 ml
RNase-free H_2O	RT	5 ml
Proteinase K	4°C	5 ml
RNase free adsorption column RA and collection tube	RT	50 sets

Description

This reagent kit is suitable for extracting free circulating nucleic acid DNA/RNA from fresh or frozen (frozen but not thawed, freeze-thaw will affect quality) serum, plasma, urine, and other cell-free body fluids. This reagent kit has developed a unique Buffer system for the small and low content of free circulating nucleic acid fragments, ensuring efficient extraction of free nucleic acid. The purified free nucleic acid has high yield and good quality, and can remove proteins, pigments, lipids, and other inhibitors to the maximum extent. This product can handle 0.1-1 ml of liquid samples, and the elution volume of the configured high-efficiency micro adsorption column can be as low as 20μl. The free nucleic acid content is extremely low (typical concentration of 1-100ng/ml plasma), and the yield is closely related to the type of sample, storage conditions, time, and individual differences. The purified free DNA has stable and reliable quality, and can be directly used in molecular biology experiments such as PCR, fluorescence quantitative PCR, and second-generation sequencing.

Features

1. No toxic reagents such as phenol and chloroform are used, and no steps such as ethanol precipitation are required.
2. Simplicity, single sample operation can generally be completed within 30-50 minutes.
3. The purified free nucleic acid has high yield and good quality, and performs better in downstream experiments.

Application

Suitable for extracting free circulating DNA/RNA and viral nucleic acid DNA/RNA from human plasma, serum, urine, or other cell-free body fluids.

Note

1. After the sample is frozen and stored until extraction, thawing should be avoided. If it is indeed unavoidable, multiple freeze-thaw cycles should be avoided, otherwise it may lead to a decrease in extraction volume.

2. It is recommended to centrifuge at 16000g for 5-10 minutes at 4℃ before treatment to further remove cell debris and reduce contamination of gDNA and RNA from damaged blood cells. If there is a small protein precipitation after freezing, it is also recommended to centrifuge at 4℃ at 16000g for 5 minutes.
3. Self prepared ethanol and isopropanol are required. Please preheat the water bath to 60℃ before starting the experiment.
4. This reagent kit can extract 0.1-1 ml of liquid samples.
5. If the sample is processed as urine, please contact us and order Buffer ATL separately.

Protocol(Please read the precautions before the experiment)

Tip: Before the first use, follow the instructions on the reagent bottle label to add ethanol to the PE bottle for protein removal and RW bottle for rinsing, and **isopropanol to the Buffer ACB bottle**. Mix well and mark the label on the reagent bottle.

1. Add 20μl Proteinase K to a 1.5ml centrifuge tube. (For samples with an initial processing volume exceeding 300μl, a larger volume suitable centrifuge tube needs to be selected.)
2. Add 200μl of serum/plasma sample.

   Note:When the sample volume exceeds 200μl, please increase the dosage of Proteinase K, Buffer ACL, and Buffer ACB reagents proportionally. The specific amount of reagents added can be referred to in the final table.
3. Add 160μl Buffer ACL, mix well, and vortex for at least 30 seconds.
4. Incubate at 60℃ for 30 minutes, invert and mix several times during this time.

   Note:Incubate 200μl serum/plasma samples at 60℃ for 10-15 minutes.
5. Add 360μl Buffer CB (**check if isopropanol is added before use**), vortex and shake for 15-30 seconds to mix well.
6. Ice bath for 5 minutes, briefly centrifuge to concentrate the liquid on the tube wall and wall cover to the bottom of the tube.
7. Immediately mix the mixture (less than 750μl each time . It can be added in two batches and then added to an adsorption column RA. The column is placed in a collection tube and centrifuged at 13000 rpm for 1 minute to discard the waste liquid.
8. Add 500μl Buffer RE, centrifuge at 13000rpm for 30 seconds, and discard the waste liquid.
9. Add 750μl Buffer RW (please check if anhydrous ethanol has been added first!), centrifuge at 13000 rpm for 30 seconds, and discard the waste liquid.
10. Add 750μl of anhydrous ethanol, centrifuge at 13000 rpm for 30 seconds, and discard the waste liquid.
11. Put the adsorption column RA back into the empty collection tube, centrifuge at 13000 rpm for 2 minutes, and try to remove ethanol as much as possible to avoid residual ethanol inhibiting downstream reactions.
12. Take out the adsorption column RA and place it in an RNase free centrifuge tube. Add 20-50μl of RNase free water in the middle of the adsorption membrane (heating in a 70-90℃ water bath beforehand can increase yield). Leave at room temperature for 2 minutes and centrifuge at 13000 rpm for 1 minute- Store eluted RNA/DNA at 20℃.

    If the final concentration of nucleic acid RNA/DNA needs to be increased, the obtained solution can be re added to the adsorption column, left at room temperature for 2 minutes, and centrifuged at 13000 rpm for 1 minute.

    If the extracted free DNA can be eluted with sterilized TE Buffer, the effect is better for long-term preservation.

Recommended reagent dosage for different plasma/serum sample sizes

Sample Volume Reagent addition amount	200μl	300μl	600μl	800μl	1000μl
Buffer ACL	160μl	240μl	480μl	640μl	800μl
Buffer ACB	360μl	540μl	1080μl	1440μl	1800μl
Proteinase K	20μl	30μl	60μl	80μl	100μl

Appendix: Extraction of Free Circulating Nucleic Acid from Urine Samples

1. Add 63μl Proteinase K to a 5ml centrifuge tube.
2. Add 500μl of urine sample.

   Note:When the sample volume exceeds 500μl, please increase the dosage of Proteinase K, Buffer ACL, and Buffer ACB

reagents proportionally.

3. Add 500μl Buffer ACL and 125μl Buffer ATL, mix well, vortex shake well for at least 30 seconds.
   Note:Add Buffer ATL at the end to ensure thorough vortex mixing. When mixed, precipitation may occur, which is a normal phenomenon. Subsequent heating steps will dissolve and do not affect the yield.
4. Incubate at 60℃ for 30 minutes, invert and mix several times during this time.
5. Add 1.8 ml of Buffer CB (**check if isopropanol is added before use**), vortex for 15-30 seconds, and mix well.
6. Ice bath for 5 minutes, briefly centrifuge to concentrate the liquid on the tube wall and wall cover to the bottom of the tube.
7. Connect to Protocol 7.