DANAGENE CIRCULATING DNA MINIKIT

Ref. 0614.1 50 preps

1.INTRODUCTION

DANAGENE Circulating DNA Minikit provides a fast, reliable and convenient method to purify high quality, high purity and inhibitor-free **cell-free circulating DNA from fresh and frozen plasma / serum samples and other body fluids from samples of 1 ml.**

A specially formulated buffer system allows circulating DNA to bind to the MicroSpin columns. Samples are lysed under denaturing conditions and then transferred to the DNA column where DNA binds and cellular debris, hemoglobin, and other proteins are washed away. High-quality DNA is eluted in nuclease-free water.

Normally the circulating DNA is highly fragmented 50-1000 bp. The degree of fragmentation depends on several parameters such as the origin of DNA (fetal, tumor, microbial DNA), health blood donor, procedure blood collection, handling and storage of the sample.

Features:

- **Efficient recovery and concentration of fragmented DNA (circulating cell-free DNA) with high input and low elution volume.**
- **Sample size: 1000 ul fresh and frozen plasma/serum and other body fluids**.
- **No organic extraction or ethanol precipitation.**
- **Removal of contaminants and inhibitors.**
- **Yield: 0.1-100 ng / ml plasma or serum. Variable because each donor and disease status.**
- **Circulating DNA purified is ready for applications such PCR o real-time PCR, microarrays and Next generation sequencing.**

Applications:

- **Biomarker research and validation for blood-based cancer detection.**
- **Ideal for detection of biomarkers in different diseases like autoimmune diseases, infection diseases, stroke, sepsis, trauma and hematologic disorders. .**
- **Analysis of fetal DNA from maternal plasma.**

2. KIT COMPONENTS

	Ref.0614.1 50 extracciones	Tª Stock
Lisis Buffer PS	55 ml	15-25ºC
Proteinase K*	2 x 100 mg	-20ºC
Desinhibition Buffer*	18 ml	15-25ºC
Wash Buffer*	10 ml	15-25ºC
Elution Buffer	2 ml	15-25ºC
MicroSpin Columns	50 units	15-25ºC
2 ml collection tubes	250 units	15-25ºC
3 ml tubes	50 units	15-25ºC

(*) These solutions must be prepared as indicated in the Preliminary Preparations section of the protocol.

Both the Lysis Buffer PS and Desinhibition Buffer contain Guanidine salts which is an irritant agent , for this reason we recommend to use gloves and glasses for its manipulation. Guanidine salts can form highly reactive compounds when combined with bleach.

Equipment and additional reagents required

- Water bath
- Votexer
- Ethanol 100 % and isopropanol
- Microcentrifuge capable of 13.000 x g
- Nuclease-free 1.5-2 ml microcentrifuge tubes

3.PROTOCOL

3.1 Collection and sample storage

Plasma/serum samples , blood should be cooled and centrifuged within one hour after blood collection.

Plasma preparation :

1. Centrifuge fresh blood at 1600-2000 x g for 10 minutes.
2. Recover the plasma care not to add the layer containing the cells.
3. Keep the plasma at -80 ° C until DNA extraction.
4. **Thaw the plasma and centrifuge at maximum speed for 5 minutes to remove possible contaminating DNA coming from residual cells. Use the supernatant for DNA extraction.**

3.2 Preliminary preparations

- Dissolve the proteinase K in **2 x 2.00 ml** in nuclease-free water and store at –20ºC. It is recommended to do several aliquots to avoid many thaw/freeze cycles. At this temperature it is stable for 1 year.
- Add **40 ml** of Ethanol 100 % to the Wash Buffer . Keep the container closed to avoid the ethanol evaporation.
- Add **10 ml** of Ethanol 100 % to the Desinhibition Buffer. Keep the container closed to avoid the ethanol evaporation.
- **Pre-heat Elution Buffer at 70ºC.**

3.3 Protocol for purification circulating cell-free DNA from 1000 ul plasma/serum and body fluids samples

1. Add **1000 µl of plasma/serum** in a microtube de 1.5 ml. **Incubate at 55ºC** for 15 minutes. **Centrifuge at maximum speed** for 5 minutes.

2. Recovery supernatant containing circulating cell-free DNA using a micropipette, avoid touching the visible pellet and add into 3 ml tube supplied . Add **1000 µl Lysis Buffer PS** + **75 µl Proteinase K . Incubate at 55ºC** for 15 minutes. Mix by inverting or shaking every 5 minutes.

3. Add **750 µl Isopropanol. Mezclar bien.**

4. **Transfer 700 µl sample to the MicroSpin column** + collection tube. The column capacity is 800 µl, so this step requires 4 times to move the entire sample.

5. **Centrifuge at 8.000 rpm for 30 seconds.**

6. **Repeat this process 3 times until you pass the whole sample by the MicroSpin column.** In the fourth step, centrifuged at 14,000 rpm.

7. Place the MicroSpin column in a new collection tube and add **500 µl Desinhibition Buffer** to the reservoir**. Centrifuge at 12.000 rpm for 60 seconds.** Remove the liquid.

8. **Add 500 µl of Wash Buffer** into reservoir of Spin column**. Centrifuge at 14.000 rpm for 60 seconds.** Remove the liquid.

9. **Centrifuge at maximum speed for 3 minutes to remove the residual ethanol.**

10. Remove the collection tube and insert the Spin Column in a 1.5 ml microtube **. Add 30 µl elution buffer (preheated at 70ºC)** into reservoir of Spin column. Dispense buffer directly onto the silica membrane. **Incubate at room temperature for 2 min.** It is very important to add the elution buffer in the center of the membrane to be completely wet.

11. **Centrifuge at 10.000 rpm for 60 seconds.** Collect 30 µl and redeposit in the center of the membrane. This increases yield.

12. **Incubate 2 minutes and centrifuge at maximum speed for 1 minute.**

The microtube now contains the circulating cell-free DNA. The total cf DNA isolated can be quantified using our **Cell-free human DNA detc-qPCR Test** designed to target a conserve sequence region of a gene repeated more than a hundred times in the human genome.
Are individuals ready-to-use tubes containing all the components needed to perfom the quantitative PCR assay.

3.4 OPTIONAL protocol for purification circulating cell-free DNA from 250 µl / 500 µl plasma/serum and body fluids samples

Thaw the plasma / serum and centrifuge at maximum speed for 5 minutes to eliminate the possible contaminating cellular DNA that comes from residual cells. Use the supernatant for DNA extraction.

1. Pipetear **250-500 µl de plasma/suero** en un microtube of 1.5 ml-3.0 ml. Add**500-1000 µl Lysis Buffer PS** + **40-75 µl Proteinase K .** Vortex and mix well.

2. **Incubate at 55ºC** for 30 minutes.

3. Add **375-600 µl of isopropanol. Mix well.**

4. **Transfer 600-700 µl of sample to the MicroSpin column +** collection tube.

5. **Centrifuge at 8.000 rpm for 30 seconds.**

6. **Repeat this process until you pass the whole sample by the MicroSpin column.**

7. Place the MicroSpin column in a new collection tube and add **500 µl Desinhibition Buffer** to the reservoir**. Centrifuge at 12.000 rpm for 60 seconds.** Remove the liquid.

8. **Add 500 µl of Wash Buffer** into reservoir of Spin column**. Centrifuge at 14.000 rpm for 60 seconds.** Remove the liquid.

9. **Centrifuge at maximum speed for 3 minutes to remove the residual ethanol.**

10. Remove the collection tube and insert the Spin Column in a 1.5 ml microtube **. Add 30 µl elution buffer (preheated at 70ºC)** into reservoir of Spin column. Dispense buffer directly onto the silica membrane. **Incubate at room temperature for 2 min.**

It is very important to add the elution buffer in the center of the membrane to be completely wet.

11. **Centrifuge at 10.000 rpm for 60 seconds.** Collect 30 µl and redeposit in the center of the membrane. This increases yield.

12. **Incubate 2 minutes and centrifuge at maximum speed for 1 minute.**

4. PROBLEM GUIDE AND POSSIBLE ANSWER

For any doubt or additional consultation on the protocol, do not hesitate to contact with the technical service of DANAGEN-BIOTED S.L info@danagen.es