DANAGENE SPIN FOOD-STOOL KIT

Ref. 0609.1 50 Preps
Ref. 0609.2 250 Preps

1.INTRODUCTION

This kit has been optimized for an efficient and fast purification of **total DNA from fresh or frozen stool samples and various food samples** (raw material and processed food).

After the samples have been homogenized, the DNA can be extracted with the extraction buffer, lysis mixtures should be cleared by centrifugation or filtration in order to remove contaminants and residual cellular debris. The clear supernatant is then mixed with the binding buffer, proteinase K and isopropanol to create conditions for optimal binding to the silica membrane column. After washing with two different buffers for efficient removal of potential PCR inhibitors, DNA can be eluted in low salt buffer or water, and is ready-to-use in subsequent reactions.

Applications:

- **DNA extracted from faecal specimens is an important tool in different areas of molecular genetic research reaching from cancer diagnostics to population genetic studies.**
- **DNA from complex matrices, processed food, soya, chocolate, cereals, meat, animal feed.**
- **Detection of genetically modified material in food products.**
- **DNA suitable for PCR, real-time PCR, Southern blotting, enzymatic reactions.**
- **Detection of specific DNA in animal feed.**

2. KIT COMPONENTS

Reagents	50 preps	250 preps	Tª Stock
CTAB Extraction Buffer	65 ml	325 ml	15-25ºC
Binding Buffer	15 ml	65 ml	15-25ºC
Proteinase K*	30 mg	2 x 75 mg	-20ºC
Desinhibition Buffer*	16.5 ml	82. 5 ml	15-25ºC
Wash Buffer*	10 ml	50 ml	15-25ºC
Elution Buffer	10 ml	50 ml	15-25ºC
Spin Columns	50 unid.	250 unid.	15-25ºC
Collection Tubes	100 unid.	500 unid.	15-25ºC

(*)**These solutions must be prepared as indicated in the section of preliminary preparations.**

2.1 Equipment and additional reagents required

- Isopropanol.
- Ethanol 100 %
- Microcentrifuge.
- 1.5 ml microtubes
- Equipment for sample disruption and homogenization and/or liquid nitrogen.
- Vortex Mixer.
- Heating block for incuabtion at 80ºC.

2.2 Storage Conditions

- All kit components can be stored at indicate temperatures and are stable for at least one year.

3. PROTOCOL

3.1 Preliminary Preparations

- Both the Lysis/ Binding Buffer and the Desinhibition Buffer contain guanidine hydrochloride, which can form reactive components when combined with bleach. Both buffers are irritating agents, for this reason we recommend the use of gloves and glasses for handling. In case of contact with skin or eyes, wash with plenty of water.
- Dissolve the proteinase K in **1.3 ml** (50 preps) or in **2 x 3.35 ml** (250 preps) of nuclease-free water and store at –20ºC. It is recommended to do several aliquots to avoid to many thaw/freeze cycles. At this temperature it is stable for 1 year.
- Verify that the Tissues Lysis Buffer do not have precipitates due to the low temperatures. If necessary, dissolve heating at 37ºC.
- Add **10 ml** (50 preps) or **50 ml** (250 preps) of Ethanol 100 % to the Desinhibition Buffer. Keep the container closed to avoid the ethanol evaporation
- Add **40 ml** (50 preps) or **200 ml** (250 preps) of Ethanol 100 % to the Wash Buffer . Keep the container closed to avoid the ethanol evaporation.
- Pre-heat the Elution Buffer at 70ºC. For some downstream applications, concentrated DNA may be required. Elution with volumes of less than 200 µl increases the final DNA concentration in the eluate significantly but slightly reduces overall DNA yield . For samples containing <3 µg of DNA, eluting the DNA in 100 µl is recommended. For samples containing less than 1 µg of DNA, only one elution in 50 µl is recommended.

3.2 Protocol for the DNA isolation from 200 mg of fresh or dry stool

NOTE: It is recommended to begin with big quantities of stool samples when the DNA is not distributed homogeneously or it is in small quantities in the sample. Samples of smaller size can be processed to the suitable overalls when it is required to eliminate to the maximum the possible inhibitors of the PCR.

In general, for good results of PCR to use the minimum quantity possible of DNA, the volume will never exceed of 10% of the final volume of the mixture of PCR. It is recommended to add BSA to a final concentration of
0.1 mg / ml of the mixture of PCR and to use HOT Star polymerase

1. Weigh **180-200 mg of stool sample** and place them in 2.0 ml microtube .**Add 1.20 ml of CTAB Extraction Buffer.** Vortex vigorously continuously for 1 minute or until the stool sample is thoroughly homogenized . In some stool samples, it must be necessary the use of homogenization with a manual electric homogenizer.

*For samples of dry stool **it is necessary** the homogenization of the sample with a manual electric homogenizer.*

If the sample is liquid transfer 200 µl into a 2.0 ml microtube.

2. Incubate at 70ºC for 30-60 minutes. Vortex several times during the incubation.

For detection of human DNA it is sufficient to incubate at 70ºC, for detection of bacterial DNA incubate at 80ºC.
For the detection of cells difficult to lyse, as some bacteria or parasites, the incubation temperature you can increase at 95ºC if it was necessary.

3. Centrifuge at 14.000 rpm for 5 minutes. A pellet will appear and in the surface a layer of fat, to introduce the pipette tip crossing this superficial layer of fat, only trying to pick up **500 µl of supernatant** that it is the transparent liquid with color (to avoid to catch pellet and superficial layer) and to place in a 1.5 ml microtube.

4. Add 25 µl of Proteinase K. Incubate at 70ºC for 10 minutes.

5. Add **250 µl of Binding Buffer** and vortex briefly.

6. Add the lysate into reservoir of a combined Spin Column–collection tube assembly. **Centrifuge at 10.000 rpm for 60 seconds.** Remove the collection tube.

7. Place the Spin column in a clean collection tube, add **500 µl of Desinhibition Buffer.Centrifuge at 12.000 rpm for 1 minute.** Discard the flow-through.

8. Add **700 µl of Wash Buffer. Centrifuge at 14.000 rpm for 1 minute.** Discard the flow-through.

9. Dry silica membrane. Centrifuge at 14.000 rpm for 3 minutes.

10. Place the Spin Column into a 1.5 mL nuclease-free tube (not provided) and add **100-200 µL Pre-heat the Elution Buffer** at 70ºC. Incubate **at room temperature** for **2 minutes**.

11. Centrifuge the spin column-tube assembly **at 14.000 rpm for 1 minute**, then discard the column. The purified DNA is in the tube.

3.3 Protocol for the DNA isolation from 200 mg food samples

Given the great variety of samples that you/they embrace the foods, vegetable origin, animal origin, processed foods, raw materials, etc, becomes difficult to present an universal protocol for all the samples. It is for this reason that the Technical Department of DANAGEN can study and to put to point its specific protocol for its certain sample type.

1. Weigh **200 mg of sample** and place them in 2.0 ml microtube .**Add 1.20 ml of CTAB Extraction Buffer + 25 µl of Proteinase K..** Vortex vigorously .

2. **Incubate at 65ºC for 30 minutes.**

The main and more important step to obtain good yield is a good homogenization of the sample that will be specific for each sample type. The lysis procedure is most effective when well homogenized, powdered samples are used. To achieve this, we recommend grinding with a pestle and mortar in the presence of liquid nitrogen or using steel beads. Commercial homogenizers can also be used.
As general norm in solid samples (sausages, etc), to prepare several fragments and to homogenize with a hand electric homogenizer ; In powdered solid samples (flours, etc.) to homogenize with a hand electric homogenizer; In solid samples of great size (corn flakes , chocolate, cookies, etc) to use a grinder of coffee to pulverize a big sample and then to weigh the required quantity of powder; In liquid samples to use 200 ml directly.

3. Centrifuge at 14.000 rpm for 5 minutes. A pellet will appear and in the surface a layer of fat, to introduce the pipette tip crossing this superficial layer of fat, only trying to pick up **500 µl of supernatant** that it is the transparent liquid with color (to avoid to catch pellet and superficial layer) and to place in a 1.5 ml microtube.

4. Add **250 µl of Binding Buffer** and vortex briefly.

5. Add the lysate into reservoir of a combined Spin Column–collection tube assembly. **Centrifuge at 10.000 rpm for 60 seconds.** Remove the collection tube.

6. Place the Spin column in a clean collection tube, add **500 µl of Desinhibition Buffer.Centrifuge at 12.000 rpm for 1 minute.** Discard the flow-through.

7. Add **700 µl of Wash Buffer. Centrifuge at 14.000 rpm for 1 minute.** Discard the flow-through.

8. Dry silica membrane. Centrifuge at 14.000 rpm for 3 minutes.

9. Place the Spin Column into a 1.5 mL nuclease-free tube (not provided) and add **100-200 µL Pre-heat the Elution Buffer** at 70ºC. Incubate **at room temperature** for **2 minutes**.

10. Centrifuge the spin column-tube assembly **at 14.000 rpm for 1 minute**, then discard the column. The purified DNA is in the tube.

4. PROBLEM GUIDE AND POSSIBLE ANSWER

For any doubts or additional questions about the protocol, please contact the technical service of DANAGEN-BIOTED S.L info@danagen.es