DANAGENE SPIN FOOD-STOOL
"Bacterias" KIT

Ref. 0608.1 50 isolations
Ref. 0608.2 250 eisolations

1.INTRODUCTION

1.1 Product Description

This kit has been optimized for an efficient and fast **PCR-ready bacterial DNA** extraction (Listeria, Salmonella, E.coli, etc) **from pre-enrichment or enrichment culture** from different **food samples**, **raw materials** or **feces** using glass fiber membrane MicroSpin columns which selectively bounds the DNA.

The process includes the centrifugation of 1 ml of pre-enrichment (peptone water) or enrichment medium for concentrating the cells. The cells are lysed during an incubation time with lysoziyme (supplied with the kit). After a digestion with Proteinase K (supplied with the kit) and cleaning the lysis mixture by centrifugation and DNA binding to the glass fiber packed in the MicroSpin columns. The bounded DNA is purified by several wasshes to remove the potential PCR inhibitors ans it is finally eluted.

1.2 Kit Components

	50 isolations	250 isolations	Tª Stock
Lysozyme Reaction Buffer	15 ml	75 ml	15-25ºC
Lysis/ Binding Buffer	15 ml	75 ml	15-25ºC
Lysozyma *	12 mg	60 mg	-20ºC
Proteinase K*	22 mg	105 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
MicroSpin Columnas	50 unid.	250 unid.	15-25ºC
Collection tubes	100 unid.	500 unid.	15-25ºC

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

ATENCION: Both the Lysis/ Binding Buffer and the Desinhibition Buffer contain Guanidine hydrochloride which is an irritant agent , for this reason we recommend to use gloves and glasses for its manipulation.

1.3 Equipment and additional reagents required

1. Isopropanol.
2. Ethanol 100 %
3. Microcentrifuge.
4. 1.5 ml microtubes

2. PROTOCOL

2.1 Prelyminary Preparations

- Dissolve the Lysozyme in **1.10 ml** (50 extractions) or in **5.50 ml** (250 extractions) of 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

- Dissolve the proteinase K in **1.10 ml** (50 extractions) or in **5.20 ml** (250 extractions) of 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.
- Verify that the Lysis/ Binding Buffer do not have precipitates due to the low temperatures. If necessary, dissolve heating at 37ºC.
- Add **10 ml** (50 extractiond) or **50 ml** (250 extractions) of Ethanol 100 % to the Desinhibition Buffer. Keep the container closed to avoid the ethanol evaporation
- Add **40 ml** (50 extractions) or **200 ml** (250 extractions) 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.

2.2 Protocol for DNA isolation from 1 ml of pre-enrichment or enrichment medium

1. Transfer 1-1.5 ml of pre-enrichment or enrichment medium to a 1.5 ml microtube and centrifuge **for 5 minutes at 12.000-14.000 rpm.**

2. Add **300 µl of Lysozyme Reaction Buffer** + **20 µl Lysozyma.** Mix well.. Incubate at 37ºC for 30 minutes.

3. Add **300 µl of Lysis/ Binding Buffer** + **20 µl Proteinase K.** Mix well. Incubate at 70ºC for 10 minutes.

4. Add **150 µl of Isopropanol.** Mix well and centrifuge 60 seconds at 14.000 rpm .

5. Pipette the lysate into reservoir of a combined MicroSpin Column –collection tube assembly. **Centrifuge at 8.000 rpm for 60 seconds.** Remove the collection tube.

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

7. Add 500 µl of Wash Buffer into reservoir of MicroSpin column**. Centrifuge at 12.000 rpm for 60 seconds.**Remove the liquid.

8. 2º Wash. **Add 500 µl of Wash Buffer** into reservoir of MicroSpin column. Centrifuge at 14.000 rpm for 60 seconds. Remove the liquid.

9. Centrifuge at maximum speed for 2 minutes to remove the residual ethanol.

10. Remove the collection tube and insert the MicrSospin column in a 1.5 ml microtube**. Add 50-200 µl of Elution Buffer** (preheated at 70ºC) into reservoir of MicroSpin column. Incubate 2 minutes.

11. Centrifuge at maximum speed for 60 seconds. The microtube contains now genomic DNA.

3. GUPROBLEM GUIDE AND POSSIBLE ANSWER

For any question regarding the work protocols o problems. Please, contact DanaGen-BioTed technical service for any comment or question regarding the protocol.