Condagene®

Condagene® Extraction Column 250 rxn

6507

Condalab

Laboratorios Conda S.A.
C/ Forja, 9. Torrejón de Ardoz
28850 Madrid, Spain
T. +34 91 761 02 00

INTRODUCTION AND PRODUCT DESCRIPTION

Condagene® Extraction Column provides an efficient procedure for the isolation of total DNA from food samples (raw material and processed food of animal or plant origin) and bacteria. After the food samples have been homogenized, the DNA can be extracted with the lysis buffer. In case to detect bacterial DNA in food samples, a pre-enrichment with the appropriate culture medium is recommended. The bacterial DNA can be extracted with the lysis buffer, after a concentration step by centrifugation.

Lysis occurs by thermal shock and the purification by a series of processes to remove contaminants and residual cellular debris. The clear supernatant is then mixed with the binding buffer, to create conditions for optimal binding to the silica membrane column. The purified DNA is ready-to-use for subsequent reactions like real-time PCR detection and quantification.

Applications:

- DNA from complex matrices, processed food, raw food and animal feed, soya, chocolate, cereals, meat
- Detection of specific DNA in animal feed
- Detection of genetically modified material in food products (OGM)
- Extraction of microbial DNA
- DNA Extraction from swabs and sponges
- DNA suitable for PCR, real-time PCR, enzymatic reactions

CONTENTS AND STORAGE

If properly stored, see the expiration date for the stability of the Kit. RT: room temperature.

Name Tube	Volume or unit		Storage
	50 preps	250 preps	
Lysis buffer	65 mL	325mL	RT
Binding buffer	13 mL	65mL	RT
Proteinase K *	30 mg	2x75 mg	-20°C
Wash buffer 1 **	16.5 mL	82.5 mL	RT
Wash buffer 2 **	10 mL	50 mL	RT
Elution	10 mL	50 mL	RT
Spin columns	50 units	250 units	RT
Collection Tubes	100 units	500 units	RT

Notes

* Reconstitute Proteinase K by adding nuclease-free water (Molecular Biology grade) as indicated on vial(s) and stored at -20°C. It is recommended to do several aliquots to avoid thaw/freeze cycles. At this temperature is stable for 1 year.

** Add ethanol (96-100%) to Wash buffers prior to use as indicated on the bottle(s). Keep the containers closed to avoid the ethanol evaporation.

MATERIAL REQUIRED BUT NOT SUPPLIED

Microcentrifuge tube (1.5 – 2.0 mL; 15 – 50 mL)
Micropipettes and micropipette filter tips (10 – 100 µL and 100 – 1000 µL) Vortex
Microcentrifuge, able to operate up to 10.000 g.
Heater block (preferably) or Water bath at 37, 65±5 °C to 95±5 °C Powder-free gloves
Ethanol 100 %
Condagene® Real Time Detection kits for allergens or pathogens (optional)

WARNINGS AND PRECAUTIONS

These products are exclusively for in vitro use

The test requires qualified staff to prevent the risk of erroneous results Do not mix reagents from different batches

Do not use reagents from other manufacturer's products

Wear disposable gloves, laboratory coats when handling specimens and reagents. Use sterile pipette tips with filters

Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately

Both the Lysis, Bindind buffers and Washing buffer 1 contain guanidine hydrochloride, which can form reactive components when combined with bleach (sodium hypochlorite)

Material Safety Data Sheets (MSDS) are available on request

Waste must be treated and disposed of in compliance with the appropriate safety standards

Clean periodically the working space with at least 5% of sodium hypochlorite

It is strongly recommended to have dedicated areas, materials, and equipment for the DNA extraction, preparation of the PCR and post-PCR procedures

KIT USAGE INFORMATION

The kit contents should be mixed slightly before use.

Under cool environmental conditions, a precipitate may form in the Lysis buffer. In this case, the component should be heated to dissolve precipitate approximately 5 minutes at 37°C and thoroughly shaken prior use.

PROCEDURE

PRELIMINARY PREPARATIONS

⚠ Make sure that Proteinase K and Wash Buffers were prepared according to "*Contents and Storage*" section, page 2

⚠ Preheat Lysis buffer and Elution buffer to 65-70°C

1. DNA ISOLATION FROM FOOD

(for dairy products see the procedure #2)

Homogenize sample

1. Weigh about **200 mg of food** material and transfer to a microcentrifuge tube
   To obtain a good yield, good homogenization of the sample is necessary. The lysis procedure is most effective when well-homogenized samples are used. We recommend grinding with a pestle and mortar or commercial homogenizers.
   In liquid samples use 200 µL directly.
   For dairy products see procedure #2 (page 6).

Lyse cells step

2. Pipette **1000** µL **of Lysis buffer** (preheated) to the microcentrifuge tube, and add **25** µL **of Proteinase K.** Vortex vigorously

3. Apply a short spin down and incubate for **30 minutes at 65°C.** Vortex one ortwice time during incubation (optional)

   For samples with hight fat content (ex: chorizo, cheese, ham, etc) an O/N lyse step (16-18h at 65°C) and the double of Proteinase K (50 ul) may be necessary.

4. Centrifuge at **>10.000 g for 5 minutes**

DNA bindin step

5. Transfer **500** µL **of clear supernatant** to a new microtube with **250** µL **ofBinding buffer**. Vortex briefly

   On the surface, a layer of fat could appear. To collect a clear supernatant, introduce the pipette tip crossing this surficial layer of fat, only trying to pick up the supernatant liquid with color (and avoid touching with the tip in the pellet).

6. Place the spin column in a 2 mL collection tube

7. Transfer **600** µL **of the mix** to the column, and centrifuge at >**10.000 g for 1minute.** Discard flow-through

8. Repeat step 7 to **load the remaining sample**

9. Place the spin column in a new collection tube

Washing step

10. Add **500 µl of Wash Buffer 1** and centrifuge at >**10.000 g for 1 minute**. Discard flow-through

11. Add **700 µl of Wash Buffer 2** and centrifuge at >**10.000 g for 1 minute**. Discard flow-through

12. Centrifuge at >**10.000 g for 3 minutes**

Elution step

13. Transfer the spin column to a new 1.5 ml microcentrifuge tube and pipette **100** µL **of Elution Buffer** (preheated at 70°C) onto the membrane. Incubateat room temperature for 2 minutes

    Decrease the volume of elution buffer (such as 50 µL)if a higher DNA conctration is desired.

14. Centrifuge at >**10.000 g for 1 minute**. Discard the spin column and use DNAimmediately or store at -20°C

2. DNA ISOLATION FROM DAIRY PRODUCTS

⚠ Before starting, preheat Lysis buffer and Elution buffer to 70°C

Homogenize sample

1. Homogenize the food sample and centrifuge about **50 mL** at >**10.000 g for 10 minutes**. Discard the supernatant
   To obtain a good yield, good homogenization of the sample is necessary. The lysis procedure is most effective when well-homogenized samples are used. We recommend this procedure for samples for dairy products such as milk, butter and cream cheese or cottage cheese. Other types of cheeses weigh 300 mg of food material and then and go directly to step 4, of this procedure.

2. Centrifuge more **10 minutes** at >**10.000 g.** Discard the supernatant

3. Wash the pellet with 1 mL of nuclease-free water or PBS. Then, centrifuge **3 minutes** at >**10.000 g**

4. Pipette **1000** µL **of Lysis buffer** (preheated) to the microcentrifuge tube, and add **50 µL of Proteinase K.** Vortex vigorously

5. Apply a short spin down and incubate for **30 minutes at 65°C.** Vortex one or twice times during incubation

   OPTIONAL: O/N lyse step (16-18h at 65°C)

6. Centrifuge at **>10.000 g for 5 minutes**

7. Proceed to DNA binding, Washing and Elution steps, in procedure #1 "DNA ISOLATION FROM FOOD" (page 5)

3. BACTERIAL DNA ISOLATION FROM ENRICHMENT CULTURE

⚠ Before starting, preheat Lysis buffer and Elution buffer to 70°C

Concentration step

1. Centrifuge **1 mL** of pre-enrichment or enrichment medium at >**10.000 g for 5 minutes**. Discarded the supernatant
   Avoid transferring food debris from the enrichment medium into the microcentrifuge tube.

Lyse cells step

2. Pipette **500** µL **of Lysis buffer** (preheated) to the microcentrifuge tube. Vortex vigorously.

3. Apply a short spin down and incubate for **15 minutes at 95°C**

4. Centrifuge at **>10.000 g for 5 minutes**

5. Proceed to DNA binding, Washing and Elution steps, in procedure #1 "DNA ISOLATION FROM FOOD" (page 5)

4. DNA ISOLATION FROM SWABS AND SPONGES

⚠ Before starting, preheat Lysis buffer and Elution buffer to 70°C

Lyse cells step

1. Add swab/sponge to **1000 µL of Lysis buffer** (preheated) to the microcentrifuge tube, and add **25 µL of Proteinase K.** Vortex vigorously

2. Incubate at Room Temperature for 2 hours with shaking

3. Take out the swab/sponge by pressing it to the wall of the microcentrifuge tube in order to recover all sample
4. Apply a short spin down and incubate for **30 minutes at 65°C.**
   Vortex one or twice time during incubation (optional)

5. Centrifuge at **>10.000 g for 5 minutes**

6. Proceed to DNA binding, Washing and Elution steps, in procedure #1 "DNA ISOLATION FROM FOOD" (page 5)

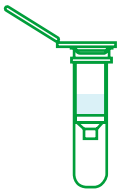
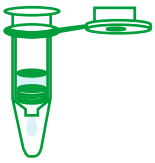
WORKFLOW (1. FOOD and 3. ENRICHMENT CULTURE)

PROCEDURE 1 and 3

1 FOOD HOMOGENIZATION		Homogenize and weight 200 mg of food (for other procedures see the complete protocol)
2 LYSE CELLS		1000 µL Lysis buffer + 25 µL Proteinase K; 65°C, 30 min (food) **or** 500 µL Lysis buffer 95°C, 15 min (bacteria) >10.000 x g, 5 minTake 500 µL of supernatant and continue with step 4
3 BIND DNA		500 µL of supernatant (step 3) 250 µL Binding buffer Load the spin column (max. 600 µL) >10.000 x g, 1 min
4 WASH		500 µL of Wash buffer 1 >10.000 x g, 1 min 700 µL of Wash buffer 2 >10.000 x g, 1 min >10.000 x g, 3 min
5 ELUTE DNA		100 µL of Elution buffer (preheated at 70°C) >10.000 x g, 1 min

WORKFLOW (2. DAIRY FOOD)

PROCEDURE 1 and 3

1 FOOD HOMOGENIZATION		Homogenize and weight 50 mL of food (for each type of food see the complete protocol)
2 WASH PELLET		>10.000 x g, 10 min >10.000 x g, 10 min 1 mL nuclease-free water (or PBS) >10.000 x g, 3 min
3 LYSE CELLS		1000 µL Lysis buffer + 50 µL Proteinase K 65°C, 30 min (food) >10.000 x g, 5 min Take 500 µL of supernatant and continue with step 4
4 BIND DNA		500 µL of supernatant (step 3) 250 µL Binding buffer Load the spin column (max. 600 µL) >10.000 x g, 1 min
5 WASH		500 µL of Wash buffer 1 >10.000 x g, 1 min 700 µL of Wash buffer 2 >10.000 x g, 1 min >10.000 x g, 3 min
6 ELUTE DNA		100 µL of Elution buffer (preheated at 70°C) >10.000 x g, 1 min

TROUBLESHOOTING

Trouble	Possible Reason	Solution Suggest
Low DNA yield or low DNA purity	Inappropriate storage conditions	The Kit should be stored between +15 and +25°C, except Proteinase K should be stored at -20°C. The tube and bottle caps must be tightly sealed after each use to maintain the pH values and stability of the kit components, and to prevent contamination
	Chemicals and sample are not mixed well	The sample should be thoroughly mixed after each chemical addition
	Poor elution	Incubating the column with elution buffer for 2 minutes at 70 °C may increase the yield Elute the DNA with 50 µL of elution buffer
No amplification after PCR/ qPCR run or the enzymatic reactions are not working	Alcohol residue in DNA isolate	The remaining ethanol after washing steps should be removed by centrifuging the column at >10000 g for 3 minute

QUALITY CONTROL

Each lot of the kit is tested against predetermined specifications to ensure consistent product quality.

TRADEMARK, DISCLAIMER AND PRODUCT USE RESTRICTION

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law. Condagene® kit handbooks and user manuals can be requested from Condalab or your local distributor.

The use of this product signifies the agreement of any purchaser or user to the following terms: The kit must be used solely in accordance with the respective Instructions for Use. Condalab grants no license under any of its intellectual property to use or incorporate the enclosed components of this Kit with any components not included within this kit except as described in the Instructions for Use. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.

The kit components are intended exclusively for in vitro use, and for research purposes only! Condalab products are intended for general laboratory use only! Molecular Biology procedures, such as DNA extractions and PCR amplification, require qualified staff to prevent the risk of erroneous results. It is strongly recommended to have dedicated areas, materials and equipment for the DNA extraction, preparation of the PCR and post-PCR procedures. The workflow in the laboratory should proceed in a uni-directional manner, from the Extraction Area to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed the previous step. The user should always read all the instructions provided with the product before running the assay. Not mix reagents from different batches. Not use reagents from other manufacturer's products. Wear disposable gloves, laboratory coats when handling specimens and reagents. Use sterile pipette tips with filters. Waste must be treated and disposed of in compliance with the appropriate safety standards.

ADDITIONAL INFORMATION

For additional information, technical support or troubleshooting please contact: micro@condalab.com

ORDERING INFORMATION

Condalab offers a large selection of products. Visit www.condalab.com or contact micro@condalab.com for more detailed product information.

Extraction Kits

Reference	Description
6500	Condagene® Extraction Complex 100 rxn
6504	Condagene® Extraction Rapid 100 rxn
6505	Condagene® Extraction Rapid 250 rxn
6506	Condagene® Extraction Column 50 rxn
6507	Condagene® Extraction Column 250 rxn

Condalene®

www.condalab.com