CONGEN

SureFood® PREP Advanced

Art. No. S1053
50 extractions

User Manual

Efficient DNA preparation from highly processed food and feed

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1 General Information

1.1 Description

This kit is intended to be used for the extraction of animal and plant DNA (deoxyribonucleic acid). It can be used with two different protocols.

The DNA extraction protocol 1 is designed for the sensitive extraction of animal and plant DNA from food according to regulation (EU) 1169/2011.

The DNA extraction protocol 2 is designed for the extraction of plant DNA from highly processed food and feed. Furthermore, it can be used for samples that produce PCR inhibiting DNA extracts when extracted with protocol 1. Potentially occurring PCR inhibiting substances are efficiently removed in additional binding and purification steps.

1.2 Kit components and storage

Kit Code	Reagent /Material	Amount
K	Proteinase K	1 Tube
H	PCR grade water	1 x 1.1 ml
L	Lysis Buffer	1 x 60 ml
B	Binding Buffer*	2 x 15 ml
P	Pre-Wash Buffer**	1 x 60 ml
W	Wash Buffer**	1 x 60 ml
E	Elution Buffer	1 x 10 ml
X	Elution Buffer X	1 x 12 ml
F	Spin Filter	1 x 50 Filter
S	Spin Filter	2 x 50 Filter
T	Receiver Tubes 1,5 ml	1 x 50 Tubes
R	Receiver Tubes 2,0 ml	4 x 50 Tubes

*After adding isopropanol (not supplied with the kit, see 1.3 additionally required equipment and materials)
**After adding ethanol (not supplied with the kit, see 1.3 additionally required equipment and materials)

The components are divided into two boxes.
After addition of water the Proteinase K should be stored at -28 to -16 °C. All other reagents of the kit should be stored dry and at room temperature (14-25 °C).
Note: After freezing and thawing of the Proteinase K flocculation may occur. Through vortexing the precipitation solves and the Proteinase K can be used again.
Before every extraction make sure that all components have room temperature. If there are any precipitates within the provided solutions, solve them by warming carefully (up to 30 °C).

1.3 Additionally required equipment and materials

- suitable equipment for sample comminution and homogenization
- micro balance and spatula for weighing the samples
- reaction tubes free from DNA and DNase 1.5 ml; 2.0 ml
- waterproof pen and tags for labeling the reaction tubes
- unpowdered disposable gloves
- pipettes with filter tips
- Vortex mixer
- Thermomixer/ heating block (up to 65°C)
- micro centrifuge (up to 12,000 rpm)
- ethanol for preparation of Wash Buffer (puriss., purity ≥ 96%)
- isopropanol for preparation of Binding Buffer (e.g. Carl Roth 2-Propanol Rotipuran® >99.7; Applichem 2-Propanol for microbiologie; Sigma-Aldrich 2-Propanol)

A flow chart for visualization of the extraction protocol is available at the CONGEN homepage: http://www.congen.de/en/downloads.

2 Protocol 1

The DNA extraction protocol 1 is designed for the sensitive extraction of animal and plant DNA from food according to regulation (EU) 1169/2011.

2.1 Principle

1. Preparation of the basic material
2. Lysis of the basic material
3. Pre-filtration and setting of optimal binding conditions
4. Binding of the nucleic acids on a Spin Filter
5. Purification of the bound nucleic acids
6. Drying of the Spin Filter
7. Elution of nucleic acids from the Spin Filter

2.2 Preparations

General

Add 1 ml PCR grade water **(Code H)** to the Proteinase K **(Code K)** and mix thoroughly.

Add 11 ml isopropanol to the Binding Buffer **(Code B)** and mix thoroughly.

Add 30 ml ethanol to the Pre-Wash Buffer **(Code P)** and mix thoroughly.

Add 42 ml ethanol to the Wash Buffer **(Code W)** and mix thoroughly.

Before each preparation

Preheating the Elution Buffer **(Code E)** - Transfer the needed amount of Elution Buffer **(Code E)** under calculation of a reserve volume into a reaction tube (not provided with the kit).

Equilibrate the Elution Buffer to 65°C. The Elution Buffer is necessary for step 7.

2.3 Extraction protocol

1. Preparation of the basic material

Transfer 100 mg* homogenized sample material into a 2.0 ml reaction tube (not provided with the kit).

* The sample amount depends on the specific sample matrix. For further questions please contact your distributor or send an e-mail to sales@r-biopharm.de.

2. Lysis of the basic material

Add 580 µl of Lysis Buffer **(Code L)** and 20 µl of Proteinase K **(Code K)** to the reaction tube and mix it briefly.

Incubate on a heating block under continuously shaking for 60 minutes at 65 °C.

Note: For strongly swelling samples it can be necessary to add additional volume of Lysis Buffer during the lysis.

3. Pre-filtration and setting of optimal binding conditions

Centrifuge the sample lysate for 1 min at 12,000 rpm.

Transfer the liquid supernatant into a new 1.5 ml reaction tube (not supplied with the kit) and centrifuge again for 1 min at 12,000 rpm.

Place a Spin Filter **(Code F – for pre-filtration)** into a 2.0 ml Receiver Tube **(Code R)**. Take 400 µl supernatant from the last centrifugation step and transfer the liquid directly onto the Spin Filter.

Centrifuge the Spin Filter with the Receiver Tube for 1 min at 12,000 rpm. After centrifugation discard the Spin Filter.

4. Binding of the nucleic acids on a Spin Filter

Add 250 µl Binding Buffer **(Code B)** to the filtrate and mix.

Place a Spin Filter **(Code S – for binding)** into a new 2.0 ml Receiver Tube **(Code R)**.

Transfer the complete solution onto the Spin Filter. Incubate at room temperature for 1 min.

Centrifuge the Spin Filter with the Receiver Tube for 1 min at 12,000 rpm. After centrifugation discard the filtrate and place the Spin Filter back into the Receiver Tube.

5. Purification of the bound nucleic acids

Add 550 µl Pre-Wash Buffer **(Code P)** to the Spin Filter and centrifuge at 1 min for 12,000 rpm.
Discard the filtrate and place the Spin Filter back into the Receiver Tube.

Add 550 µl Wash Buffer **(Code W)** to the Spin Filter and centrifuge at 1 min for 12,000 rpm.
Discard the filtrate and place the Spin Filter back into the Receiver Tube.

Once more add 550 µl Wash Buffer **(Code W)** to the Spin Filter and centrifuge at 1 min for 12,000 rpm. Discard the filtrate and place the Spin Filter back into the Receiver Tube.

6. Drying of the Spin Filter

Remove the residual ethanol by final centrifugation for 2 min at 12,000 rpm.

7. Elution of nucleic acids from the Spin Filter

Place the Spin Filter into a 1.5 ml Receiver Tube **(Code T)**.

Add 50 µl of the preheated (65 °C) Elution Buffer **(Code E)** directly onto the Spin Filter.

Incubate on a heating block for 3 min at 65 °C (no shaking).

Centrifuge for 1 min at 10,000 rpm. After centrifugation discard the Spin Filter.

The eluted DNA is ready-to-use for the PCR. The DNA can be stored for up to 24 hours at +4°C. For a storage time of more than 24 hours it should be kept at -20 °C.

3 Protocol 2

The DNA extraction protocol 2 is designed for the extraction of plant DNA from highly processed food and feed. Furthermore, it can be used for samples that produce PCR inhibiting DNA extracts when extracted with protocol 1. Potentially occurring PCR inhibiting substances are efficiently removed in additional binding and purification steps.

3.1 Principle

1. Preparation of the basic material
2. Lysis of the basic material
3. Pre-filtration and setting of optimal binding conditions
4. Binding of the nucleic acids on a Spin Filter
5. Purification of the bound nucleic acids
6. Drying of the Spin Filter
7. First Elution of nucleic acids from the Spin Filter
8. Repeated setting of optimal binding conditions
9. Second binding of the nucleic acids on a Spin Filter
10. Second purification of the bound nucleic acids
11. Drying of the Spin Filter
12. Elution of nucleic acids from the Spin Filter for analysis

3.2 Preparations

General

Add 1 ml PCR grade water **(Code H)** to the Proteinase K **(Code K)** and mix thoroughly.

Add 11 ml isopropanol to the Binding Buffer **(Code B)** and mix thoroughly.

Add 30 ml ethanol to the Pre-Wash Buffer **(Code P)** and mix thoroughly.

Add 42 ml ethanol to the Wash Buffer **(Code W)** and mix thoroughly.

Before each preparation

Preheating the Elution Buffer X **(Code X)** and Elution Buffer **(Code E)** - Transfer the needed amount under calculation of a reserve volume of Elution Buffer X **(Code X)** and Elution Buffer **(Code E)** into a reaction tube (not provided with the kit).

Equilibrate the Elution Buffer X and Elution Buffer to 65 °C. The Elution Buffer X is necessary for step 7 and Elution Buffer is necessary for step 12.

3.3 Extraction protocol

1. Preparation of the basic material

Transfer 150 mg* homogenized sample material into a 2.0 ml reaction tube (not provided with the kit).

* The sample amount depends on the specific sample matrix. For further questions please contact your distributor or send an e-mail to sales@r-biopharm.de.

2. Lysis of the basic material

Add 580 µl of Lysis Buffer **(Code L)** and 20 µl of Proteinase K **(Code K)** to the reaction tube and mix it briefly. Incubate on a heating block under continuously shaking for 30 minutes at 65°C.

Note: For strongly swelling samples it can be necessary to add additional volume of Lysis Buffer during the lysis.

3. Pre-filtration and setting of optimal binding conditions

Centrifuge the sample lysate for 1 min at 12,000 rpm.

Place a Spin Filter **(Code F – for pre-filtration)** into a 2.0 ml Receiver Tube **(Code R)**.
Take 400 µl supernatant from the last centrifugation step and transfer the liquid directly onto the Spin Filter.

Centrifuge the Spin Filter with the Receiver Tube for 1 min at 12,000 rpm. After centrifugation discard the Spin Filter.

4. Binding of the nucleic acids on a Spin Filter

Add 250 µl Binding Buffer **(Code B)** to the filtrate and mix the sample.

Place a Spin Filter **(Code S – for binding)** into a new 2.0 ml Receiver Tube **(Code R)**.

Transfer the complete solution onto the Spin Filter. Incubate at room temperature for 1 min.

Centrifuge for 1 min at 12,000 rpm. Discard the filtrate and place the Spin Filter back into the Receiver Tube.

5. Purification of the bound nucleic acids

Add 550 µl Pre-Wash Buffer **(Code P)** to the Spin Filter and centrifuge at 1 min for 12,000 rpm.
Discard the filtrate and place the Spin Filter back into the Receiver Tube.

Add 550 µl Wash Buffer **(Code W)** to the Spin Filter and centrifuge at 1 min for 12,000 rpm.
Discard the filtrate and place the Spin Filter back into the Receiver Tube.

6. Drying of the Spin Filter

Remove the residual ethanol by centrifugation for 2 min at 12,000 rpm.

7. Elution of nucleic acids from the Spin Filter

Place the Spin Filter into a new 2.0 ml Receiver Tube **(Code R)**.

Add 200 µl of the preheated (65 °C) Elution Buffer X **(Code X)** directly onto the Spin Filter.

Incubation on a heating block for 3 min at 65 °C (no shaking).

Centrifuge for 1 min at 10.000 rpm. After centrifugation discard the Spin Filter.

8. Repeated setting of optimal binding conditions

Add 200 µl of Lysis Buffer **(Code L)** and 200 µl of Binding Buffer **(Code B)** to the 200 µl filtrate from step 7 and mix thoroughly.

9. Second binding of nucleic acids on a Spin Filter

Place a new Spin Filter **(Code S – for binding)** into a new 2.0 ml Receiver Tube **(Code R)**.
Transfer the complete solution from step 8 onto the Spin Filter. Incubate at room temperature for 1 min.

Centrifuge for 1 min at 12,000 rpm. Discard the filtrate and place the Spin Filter back into the Receiver Tube.

10. Second purification of the bound nucleic acids

Add 550 µl Pre-Wash Buffer **(Code P)** to the Spin Filter and centrifuge at 1 min for 12,000 rpm.
Discard the filtrate and place the Spin Filter back into the Receiver Tube.

Add 550 µl Wash Buffer **(Code W)** to the Spin Filter and centrifuge at 1 min for 12,000 rpm.
Discard the filtrate and place the Spin Filter back into the Receiver Tube.

11. Drying of the Spin Filter

Remove the residual ethanol by final centrifugation for 2 min at 12,000 rpm.

12. Elution of nucleic acids from the Spin Filter for analysis

Place the Spin Filter into a new 1.5 ml Receiver Tube **(Code T)**.

Add 50 µl of the preheated (65 °C) Elution Buffer **(Code E)** directly onto the Spin Filter.

Incubation on a heating block for 3 min at 65 °C (no shaking).

Centrifuge for 1 min at 10,000 rpm. After centrifugation discard the Spin Filter.

Note: The DNA can also be eluted with a higher volume (100 - 200 µl) of Elution Buffer, depending on the expected yield of DNA.

The eluted DNA is ready-to-use for the PCR. The DNA can be stored for up to 24 hours at +4°C. For a storage time of more than 24 hours it should be kept at -20 °C.

4 Further Information

4.1 Product Information

- Flow chart (Download: www.congen.de/en/downloads)
- Validation Report upon request
- Product-related documents (Download: www.congen.de/en/eifu/)

4.2 Technical Support

For further questions please contact your distributor or send an e-mail to sales@r-biopharm.de.

4.3 Distribution and Ordering

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5 Safety information

Lysis Buffer

Warning
H319-H412
P264-P273-P280-P305+P351+P338-P337+P313-P501

Pre-Wash Buffer

Warning
H302-H315-H319
P264-P270-P280-P301+P312-P302+P352-P305+P351+P338-P321-P330-P332+P313-P337+P313-P362+P364-P501

Proteinase K

Danger
H315-H319-H334-H335
P261-P264-P271-P280-P284-P302+P352-P304+P340-P305+P351+P338-P312-P501

H302:	Harmful if swallowed.
H315:	Causes skin irritation.
H319:	Causes serious eye irritation.
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335:	May cause respiratory irritation.
H412:	Harmful to aquatic life with long lasting effects.
P261:	Avoid breathing dust/fume/gas/mist/vapours/spray.
P264:	Wash hands, forearms and face thoroughly after handling.
P270:	Do not eat, drink or smoke when using this product.
P271:	Use only outdoors or in a well-ventilated area.
P273:	Avoid release to the environment.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P284:	Wear respiratory protection.
P301+P312:	IF SWALLOWED: Call a POISON CENTRE or doctor if you feel unwell.
P302+P352:	IF ON SKIN: Wash with plenty of water.
P304+P340:	IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P305+P351+P338:	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P312:	Call a POISON CENTRE or doctor if you feel unwell.
P321:	Specific treatment (see supplemental first aid instruction on this label).
P330:	Rinse mouth.
P332+P313:	If skin irritation occurs: Get medical advice/attention.
P337+P313:	If eye irritation persists: Get medical advice/attention.
P362+P364:	Take off contaminated clothing and wash it before reuse.
P501:	Dispose of contents/container to hazardous or special waste collection point, inaccordance with local, regional, national and/or international regulation.

For further information we offer a Material Safety Data Sheet, see at www.congen.de/en/eifu/.
Alternatively please contact your distributor or send an e-mail to sales@r-biopharm.de.