DNA*Extractor* Gelatin

KIT FOR THE MANUAL EXTRACTION OF HIGH-QUALITY DNA FROM GELATIN

5224701001	DNA*Extractor* Gelatin

For 50 extractions

GOLD STANDARD DIAGNOSTICS

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1 INTRODUCTION

DNA*Extractor* Gelatin is designed for the extraction of high-quality and pure DNA from pure gelatin samples.

Gelatin is a highly processed product, which generally contains low amounts of DNA. Therefore, the DNA*Extractor* Gelatin kit starts from a large amount of sample material and uses a magnetic bead DNA binding method to increase DNA yield.

The extracted DNA can be used for all kinds of subsequent analytical methods, e.g. real-time and conventional PCR testing.

Please note that the kit does not contain the solvents necessary for extraction due to freight security reasons.

1.1 Intended Use

DNA*Extractor* Gelatin is designed for the manual extraction of genomic DNA from gelatin samples.

The kit is intended to be used by analytical laboratories for food and feed testing and research purposes.

The kit should be regarded as “professional use only” and should only be used by trained personal.

1.2 Test Principle

The DNA isolation process of the DNA*Extractor* Gelatin kit is based on phenol-chloroform extraction and magnetic beads nucleic acid purification technology.

After homogenisation of the sample, lysis is performed in the presence of a lysis buffer system, optimised for gelatin samples, and proteinase K. The extraction is then carried out using phenol-chloroform where the DNA remains in the aqueous phase whereas other components of the sample go into the organic phase. After separation of the aqueous phase the released DNA is bound to magnetic beads, followed by two washing steps using differently designed wash buffers to remove contaminants and interfering ions that could inhibit the subsequent applications. Afterwards, the DNA is solved in low salt buffer to be ready-to-use for further analysis or amplification. Further cleaning of the DNA may well be required, e.g. with DNA Cleaning Columns (cat. no. 5224700310).

1.3 Components of the Kit

Table 1. Components of the kit for 50 extractions

Description	**1 x 50**	**Storage**	**Shipment**
Lysis buffer Gelatin	260 mL	Store at 18°C - 25°C**	2 – 8°C
Proteinase K (20 mg/mL)	7 x 750 µL	Store at –20 °C	-20°C
Lysis Buffer EFG	4 x 50 mL	Store at 18°C - 25°C**	2 – 8°C
Magnetic Beads (50 mg/mL)	1 mL	Store at 2 – 8°C	2 – 8°C
Magnetic Beads Wash Buffer A	16 mL	Store at 18°C - 25°C**	2 – 8°C
Magnetic Beads Wash Buffer B	16 mL	Store at 18°C - 25°C**	2 – 8°C
100x TE stock buffer*	20 µL	Store at -20°C	-20°C

** (1000 mM Tris-Cl, 100 mM EDTA, pH 8.0)*

*** store at 18-25°C upon receipt*

Note: Shelf life is indicated on the labels of the kit/components.

Note: Volumes as indicated above are calculated with a defined overfilling and may vary.

Note: Avoid frequent thawing and freezing, e.g. by producing aliquots.

1.4 Additional Reagents, Consumables and Equipment Required

Table 2. Additional materials required, not provided in the kit

Reagents	• Ethanol p.a. (abs.) • Chloroform p.a. • Isopropanol p.a. (abs.) • Water, DNase-free • Phenol-chloroform-isoamyl alcohol (25:24:1)
Consumables	• Reaction tubes, 50 mL • Reaction tubes, 1.5 mL • Filter tips, check compatibility with pipettes utilised • Adequate gloves, powder free • Glass pipette for phenol • 10 mL pasteur pipette • For optional purification step: DNA Cleaning Columns (cat. no. 5224700310)
Equipment	• Centrifuge for 50 mL tubes (> 4 000 x g, 18 – 25 °C) • Micro-centrifuge for 1.5 mL tubes (> 10 000 x g, 18 – 25 °C) • Blender/Vortex mixer • Thermo shaker (up to 60 °C) • Magnetic stands for 50 mL tubes • Magnetic stands for 1.5 and 2 mL tubes • Micropipettes (variable 1 – 1 000 µL)

1.5 Related Products

- **DNA*Extractor* Fat**, cat. no. 5224700710

  Kit for the extraction of DNA from oil, fat and emulsifier (lecithin)
  for 50 DNA extractions

- **DNA*Extractor* Honey**, cat. no. 5224700910

  Kit for the extraction of DNA from honey and pollen
  for 50 DNA extractions

- **iMAGo Food**, cat. no. 5524421001

  Test kit for automated isolation of high-quality DNA from food and feed samples,
  for 1 x 96 isolations - for 1 g sample input

- **GENE*Spin***, cat. no. 5224400605

  Kit for isolation of high-quality DNA from food and feed samples,
  for 50 DNA extractions

- **DNA Cleaning Columns**, cat. no. 5224700310

  100 DNA cleaning columns, pre-packed with Sephacryl® resin and equilibrated in TE buffer (pH 7.6)

2 HOW TO USE THIS PRODUCT

Important notes:

- Store all reagents as indicated in section 1.3 after the first use.
- Do not use the reagents beyond the expiration dates printed on the labels.
- Never store kit components in the vicinity of samples or post-PCR products.
- Do not mix components of different kit lots.

2.1 General and Safety Precautions

- Relevant national health and safety regulations must be adhered to.
- Do not eat, drink or apply cosmetics in the work area.
- Do not pipette by mouth.
- Avoid contact of kit components with injured skin.
- Always wear respective protective equipment when working with chemicals
- The DNA*Extractor* Gelatin kit contains enzymes (proteinase K) which may cause allergic reactions. Buffer EFG contains ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA) and guanidine thiocyanate. Magnetic Beads Wash Buffer A contains guanidine thiocyanate and polyethylene glycol tert-octylphenyl ether.

  For more information, please refer to the respective safety data sheet (SDS).

2.2 Working Guidelines

- All work steps for preparing the test sample for subsequent analysis of GMO have to be in line with ISO 24276:2013.
- Use filter-plugged pipette tips in order to avoid possible cross-contamination.
- Use DNA-, nuclease-free and sterile lab ware.
- Use appropriate gloves and change them frequently during the process.
- After the work is completed clean the working area with Roti® Nucleic Acid-free or 1% HCl to remove DNA/ RNA contamination.
- **Please note:** Accidental contamination with DNA can be caused by dust and the spread of aerosols. Therefore, there should be a systematic delimitation of the work steps and areas based on the forward flow principle (for more details, refer to ISO 24276:2013).

2.3 Waste Disposal

For disposal of reagents and chemicals please refer to the respective safety data sheet (SDS).

2.4 Before you Begin

- If working with the DNA*Extractor* Gelatin kit for the first time, read "Working Guidelines" (see section 2.2).
- For additional reagents, consumables and equipment refer to section 1.4.
- Warm up the reagents for DNA extraction to room temperature (18 °C – 25 °C).
- Heat up thermo shaker to 60 °C in time.

2.4.1 Preparation of Reagents

0.1x TE buffer

- Dilute 100x TE stock buffer 1:1000 with DNase-free water to a final concentration of 0.1x.
- Mix well by shaking/swirling/inverting.

Magnetic beads wash buffer A and B

Before using the kit for the first time, prepare magnetic beads wash buffer A and B (each provided as concentrate) by adding the respective amount of ethanol (abs.), as indicated in table 3.

Table 3. Preparation of magnetic beads wash buffers

Component	Ethanol (abs.) to be added
Magnetic beads wash buffer A	16 mL
Magnetic beads wash buffer B	16 mL

- Mix well by shaking/swirling/inverting.
- Mark the bottles after addition of ethanol.
- Store tightly closed reagent bottles (to prevent evaporation of ethanol) at 18 – 25 °C.

2.4.2 Preparation of Samples

Sample Homogenisation

Measures have to be taken to guarantee, that the test portion (weight in for extraction) is representative for the laboratory sample (sample sent to the lab). If the laboratory sample cannot be entirely taken for milling or homogenisation, a representative analytical sample has to be taken thereof. This samples have to be milled and/or homogenised prior of taking the test portion (which has to be big enough to be representative) for extraction.

A good homogeneity of the sample is essential for an efficient and reproducible extraction. Thereby the milling and/or homogenisation procedure strongly depends on the material analysed.

During homogenisation special care has to be taken to avoid contamination. The devices chosen for crushing and milling should be easy to clean.

2.5 Extraction of Genomic DNA

The extraction of DNA from the sample is a crucial step, and in order to guarantee optimal quantity and purity, different methods are to be used depending on the sample matrix.

This kit and the associated protocol (see section 2.5.2) is optimised for gelatin samples.

If compliance with ISO 21571 is required, each sample should be extracted in duplicate.

If not mentioned otherwise, all steps are carried out at 18°C - 25°C.

2.5.1 Extraction Controls

In order to monitor the absence of environmental contaminants as well as in order to monitor the isolation efficiency the following controls are recommended (ISO 21571:2013):

- Negative extraction control: control without sample, shall always be the last in a row of samples
- Positive extraction control: a reference material with known DNA yield and quality, shall always be done when a new kit lot is used

These controls should be included in each isolation procedure.

2.5.2 Sample Processing

1. Weigh 4 g homogenised sample material into 50 mL tubes.
2. Add 5 mL lysis buffer gelatin and 50 µL proteinase K.
3. Use buffer and reagents without sample material respectively as a negative and extraction controls.
4. Overnight incubation at 60°C.
   (The sample may initially be viscous or solid, but will become liquid with the incubation at 60°C.)

2.5.3 Phenol-Chloroform Purification

Safety Precaution:

All work must be done under the fume hood. Phenol, chloroform, Buffer EFG and Magnetic Beads Wash Buffer A must be disposed of adequately and according to national regulations.

1. In order to prevent contamination of the stock, transfer 5 mL of phenol-chloroform-isoamyl alcohol mixture with a glass pipette from the stock bottle to a 50 mL tube first and avoid transferring the upper (buffer) phase. From this tube, pour the phenol-chlorform-isoamyl alcohol into the sample tube. Afterwards, vortex shortly.
2. Centrifuge for 10 min. at 10 000 x g.
3. Add 3 mL chloroform into a new 50 mL tube for every extraction.
4. Transfer the upper phase of the sample tube to the 50 mL tube prepared with 3 mL chloroform and vortex.
5. Dispose of the remaining content of the 50 mL tube into a phenol waste container.
6. Centrifuge for 5 min. at 10 000 x g.
7. Estimate the volume of the upper phase (approx. 4 mL).
8. Prepare 1 volume (approx. 4 mL) buffer EFG, 1 volume (approx. 4 mL) ethanol and 20 µL magnetic beads in a fresh 50 mL tube.

   Note: Do not use smaller volumes than those instructed above. Rather estimate the volume generously.
9. Transfer the upper phase of the sample to the prepared 50 mL tube with buffer, ethanol and beads and mix.
10. Incubate for 10 min at 18 °C – 25 °C with occasional inverting of the tube

2.5.4 DNA Purification

1. Place the tube in the magnetic stand for 50 mL tubes for approx. 10 min until all beads adhere to the wall towards the magnets.
2. Remove the supernatant with a 10 mL pasteur pipette and discard.
3. Remove the tube from the magnetic stand and resuspend the beads in 600 µL of the magnetic beads wash buffer A by pipetting up and down.
4. Transfer the resuspended beads into a 1.5 mL tube.
5. Place the tube on the magnetic stand for 1.5 mL tubes for approx. 3 min until the lysate has cleared.
6. Dispose of the supernatant.
7. Remove the tube from the magnetic stand and resuspend the beads in 600 µL magnetic beads wash buffer B by pipetting up and down.
8. Place the tube on the magnetic stand for approx. 3 min until the lysate has cleared.
9. Remove the supernatant and discard.
10. Remove the tube from the magnetic stand and wash the beads with 600 µL isopropanol.
11. Place the tube on the magnetic stand or approx. 3 min until the lysate has cleared.
12. Remove of the isopropanol completely (i.e. centrifuge the tube shortly and remove all remaining isopropanol).
13. Let the beads dry for 10 min at 60 °C with open lid.
14. Add 50 µL 0.1 x TE buffer and resuspend the beads.
15. Incubate for 5 min at 18 °C – 25 °C.
16. Place the tube with beads on the magnetic stands and transfer the supernatant with the dissolved DNA to a new 1.5 mL tube.

    Optional purification step:

    For most gelatin samples, the DNA can directly be used for PCR analysis. In case inhibition occurs and further cleaning of the DNA is necessary, our DNA Cleaning Columns (cat no. 5224700310) can be used.

2.5.5 Quality Assessment of DNA Yielded

To verify the amount of isolated DNA, a concentration measurement shall be performed. This can be done with several methods e.g.

- UV- spectrophotometric methods (very sensitive to inhibitors and therefore not very accurate)
- Quantification via real-time PCR
- Fluorescence method using intercalating dye (e.g. SYBR® Green, Midori Green)
- Capillary electrophoresis

Depending on the sample type the DNA yield might be too low to be measured, but amplification may still be able to detect the target gene region.

3 TROUBLESHOOTING

Observation:

DNA is degraded

Possible Cause	Solution
Sample contamination with DNase	**Check working area and pipettes** and clean, if necessary
Due to a high degree of processing of the sample, the DNA present in the sample may have been sheared and degraded	If possible analyse unprocessed starting material

Observation:

DNA purity is poor

Possible Cause	Solution
DNA contaminants are not fully removed	Perform further cleaning of the DNA extract, e.g. using DNA Cleaning Columns (cat. no. 5224700310)

4 PRODUCT WARRANTIES, SATISFACTION GUARANTEE

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5 IMPORTANT NOTES

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6 REVISION CONTROL

Version	Date	Changes
V2.1	10.07.2023	• Adaptation to Gold Standard Diagnostics layout for DNA Extraction kits • Harmonisation within the DNA Extraction kits • No product or assay-specific changes

TECHNICAL SUPPORT SERVICE

For technical assistance and more information, please contact Gold Standard Diagnostics Budapest's customer service or your local distributor.

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