Instruction for Use
GENE*Spin*

KIT FOR ISOLATION OF HIGH-QUALITY DNA FROM FOOD AND FEED SAMPLES

Cat. No. 5224400605

For 50 DNA extractions

GOLD STANDARD DIAGNOSTICS

Table of Contents

Kits, their components and instructions for use are subject to alterations.

1. GENESPIN - INTRODUCTION

GENESpin provides an optimal lysis and DNA extraction system for nearly all types of food samples. Resulting eluates can be used for all kinds of subsequent detection methods, e.g. real-time and conventional PCR (for very difficult sample types causing PCR inhibition, additional use of our DNA Cleaning Columns, cat. no. 5224700310, is recommended).

Gold Standard Diagnostics is a producer of test kits for food and feed analysis offering DNA extraction kits and test kits for genetically modified organisms (GMOs), food pathogens, plant and animal species.

GENESpin silica membrane spin technology allows fast and effective purification of nucleic acids from various matrices.
The silica membranes are optimized for high DNA recoveries and low binding efficiencies for impurities. For further information regarding DNA purification, please feel free to contact us.

2. KIT CONTENTS OF GENESPIN

GENESpin Lysis Buffer, 100 mL
GENESpin Binding Buffer, 30 mL
GENESpin Wash Buffer 1, 30 mL
GENESpin Wash Buffer 2, 12 mL
GENESpin Elution Buffer, 13 mL
GENESpin Proteinase Buffer, 1.8 mL
GENESpin Proteinase K, lyophilized, 6 mg
50 GENESpin Columns 9. 200 Collection tubes (2 mL)

Attention:
GENESpin Binding Buffer and GENESpin Wash Buffer 1 contain guanidinium hydrochloride and/or detergents! Wear gloves and goggles!
Guanidinium hydrochloride can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT mix bleach or acidic solutions with sample preparation waste!.

3. MATERIALS NOT CONTAINED IN THE KIT

- 96 – 100% ethanol
- 1.5 mL microcentrifuge tubes
- Pipet tips

4. EQUIPMENT NEEDED

- Water bath or incubator for 65°C and 70°C
- Heating block for 65°C
- Pipettors
- Microcentrifuge
- Vortex mixer
- Equipment for sample disruption/ preparation/homogenization

5. PRODUCT DESCRIPTION

•GENESpin is designed for the isolation of genomic DNA from food and feed samples of plant and animal origin.
•GENESpin allows processing of up to
200 mg material (larger amounts are possible through upscaled use of lysis buffer). Typical yields for GENESpin are in the range of 0.1-
10 µg DNA, however, are sample-dependent.
•The eluted DNA is ready for use in subsequent reactions like PCR or real-time PCR. (For very demanding matrices, additionally use DNA Cleaning Columns, # 5224700310.)

Sample material: 200 mg (or upscaled)
Fragment size: 300 bp – approx. 50 kbp
Binding capacity: 30 µg
Typical DNA yield: 0.1 10 µg
A260/A280: 1.6 – 1.9
Elution volume: 100 µL
Preparation time: 30 min/6 preps.

6. THE BASIC PRINCIPLE

DNA is extracted from homogenized food samples with a lysis buffer containing chaotropic salts, denaturing agents and detergents. The standard isolation procedure ensures lysis with GENESpin Lysis Buffer. Lysis mixtures are cleared by centrifugation or filtration in order to remove contaminations and residual cellular debris. The clear supernatant is mixed with the GENESpin Binding Buffer and ethanol to create conditions for optimal binding to the GENESpin silica membrane, which was selected for this purpose due to its unique DNA binding properties.

After washing with two different buffers for efficient removal of potential PCR inhibitors, DNA is eluted in low salt buffer or water and is ready-to-use for subsequent analysis or amplification.

Food samples are very heterogeneous and contain many different compounds like fat, cocoa or polysaccharides which can lead to suboptimal extraction or subsequent analysis of DNA.

GENESpin guarantees good recovery rates also for small genomic DNA fragments < 1 kb from processed, complex food matrices (e.g. ketchup or spice) with very low DNA content and degraded DNA.
In consequence, it is recommended to design primers for amplification of short DNA fragments (80-150 bp) for subsequent PCR.

7. STORAGE AND HOMOGENIZATION OF SAMPLES

The lysis procedure is most effective with well homogenized samples with small particle size. Suitable methods include grinding with pestle and mortar in the presence of liquid nitrogen or with steel beads, as well as any type of commercial homogenizer, bead mill etc. After homogenization and treatment of the sample with GENESpin Lysis Buffer, the lysate can be cleared by centrifugation or filtering.

Methods for sample homogenization/ grinding Mortar and pestle in the presence of liquid nitrogen
•Commercial homogenizers, e.g. bead mills
•VA steel beads (7 mm diameter, 4-5, beads in 15 ml tube): Chill the tube in liquid nitrogen. Vortex about 30 s. Repeat procedure until the entire sample is ground to powder. Remove the beads gently with a magnet. Keep the material frozen throughout the whole procedure. Do not use nitrogen inside the tube to avoid sticking of sample material to the beads.

8. ELUTION PROCEDURES

It is possible to adapt elution method and volume of GENESpin Elution Buffer to the subsequent application as follows:

•Complete yields: 90-100% of bound nucleic acids can be eluted by performing two elution steps with 2 x 100 µL. Combine eluates and measure yield.

•Highly concentrated eluates: With minimal elution volumes (25 - 50 µL), 60 - 80% of bound nucleic acids can be eluted, resulting in highly concentrated eluates. GENESpin Elution Buffer can be replaced by TE buffer or water (adjust pH to 8 - 8.5).

9. PREPARATION AND STORAGE OF WORKING SOLUTIONS

Store kit at room temperature (18 - 25°C) before first use. Storage at 4°C is possible but may cause precipitation of salts in buffers. If precipitations occur, warm buffer to 25 - 37°C to dissolve the precipitate before use.

Prepare the following reagents before first use:

•**GENE*Spin* Wash Buffer 2:** Add 48 mL of 96-100% ethanol to GENE*Spin* Wash
Buffer 2, mark the label of the bottle to indicate that ethanol was added. Store at room temperature for up to one year.

•**GENE*Spin* Proteinase K**: Before the first use of the kit, add 600 µL of GENE*Spin* Proteinase Buffer to dissolve the lyophilized GENE*Spin* Proteinase K. Proteinase K solution is stable for 6 months at - 20°C.

10. PROTOCOL FOR FOOD SAMPLES

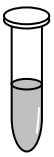
1 Homogenize samples

Homogenize about 0.2 g material with a commercial homogenizer.

homogenize samples

2 Lyse cells

Transfer the resulting powder to a 2 mL collection tube. Preheat GENE*Spin* Lysis Buffer to 65°C immediately before use and add 550 µL GENE*Spin* Lysis Buffer (65°C). Mix carefully (15 s), add 10 µL GENE*Spin* Proteinase K and mix again (2-3 s).
If the lysis buffer volume is not large enough to dissolve the sample completely, add more buffer (and Proteinase K proportionally) until the sample is totally resuspended.
Incubate at 65°C for 30 min.



*+ 550 µL GENE*Spin Lysis Buffer (65°C)*

+ 10 µL Prot. K

65°C 30 min

Optional: add 10 µL RNase A (20 mg/ml) per 550 µL lysis buffer, mix well, incubate for 30 min at room temperature.

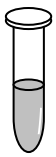
*(optional: + 10 µL RNase A per 550 µL GENE*Spin Lysis Buffer, RT 30 min)*

Afterwards, centrifuge the mix for 10 min (> 10.000x g) to pellet contaminants and cell debris.

10 min > 10.000 x g

3 Adjust DNA binding conditions

Transfer the clear supernatant into a new centrifuge tube capable of holding at least 3 sample volumes. Add 1 volume GENE*Spin* Binding Buffer and 1 volume ethanol (e.g. for 300 µL sample: add 300 µL binding buffer + 300 µL ethanol).
Vortex the mixture for 30 sec.



+ 1 vol. GENESpin Binding Buffer and +1 vol. ethanol

vortex 30 s

4 Bind DNA

For each sample, place a GENE*Spin* Column into a new 2 mL collection tube and pipette 700 µL mixture onto the column. Centrifuge for 1 min at 11.000 x g Discard flow-through. Repeat the procedure with remaining sample.

load sample (700 µL)

1 min 11.000 x g

5 Wash and dry

1st washing step

Pipette 400 µL GENE*Spin* Wash Buffer 1 onto the GENE*Spin* C11.000olumn. Centrifuge for 1 min at x g. Discard flow-through.

+ 400 µL
GENE*Spin*
Wash Buffer 1

1 min
11.000 x g

2nd washing step

Pipette 700 µL GENE*Spin* Wash Buffer 2 onto the GENE*Spin* Column. Centrifuge for 1 min at 11.000 x g. Discard flow-through.

+ 700 µL
GENE*Spin*
Wash Buffer 2

1 min
11.000 x g

3rd washing step

Pipette 200 µL GENE*Spin* Wash Buffer 2 onto the GENE*Spin* Column. Centrifuge for 2 min at remove Wash Buffer 2 min at 11.000 x g in order to remove Wash Buffer 2 completely (Residual ethanol from Wash Buffer 2 may inhibit enzymatic reactions). Discard flow-through.

+ 200 µL
GENE*Spin*
Wash Buffer 2

2 min
11.000 x g

6 Elute DNA

Place the GENE*Spin* Column in a **new** 1.5 mL centrifuge tube.

new 1.5 mL tube

Pipette 100 µL GENE*Spin* Elution Buffer (preheated to 70°C) onto the membrane.

+ 100 µL
GENE*Spin*
Elution Buffer
(70°C)

Incubate for 5 min at room temperature (18 - 25°C).

5 min RT

Centrifuge for 1 min at 11.000 x g to elute the DNA.

1 min
11.000 x g

10.1 Important hints and remarks

Considering the generally rather low DNA content in processed food, this protocol should be started with at least 200 mg of sample material.

Lysis buffer was tested for extraction of DNA from various types of samples including food of plant and animal origin.

RNase A use

RNase A (not included in the kit) addition is recommended for RNA-rich samples (i.e. unprocessed samples of plant or animal origin). Add 10 µL of 20 mg/mL stock solution (or equivalent amount) per 550 µL lysis buffer in step 2 or perform a RNase A digestion in the eluate before further use.

A vacuum manifold can optionally be used for acceleration of washing steps. Loading and elution steps should be done with centrifugation as described in the protocol.

10.2 Special hints for difficult matrices

- Ketchup, sauce and similar fluid samples:
  (0.2 g equivalents) can be mixed with GENESpin Lysis Buffer, 500-1000 µL each, and incubated with GENE*Spin* Proteinase K as described in the protocol.
- Powdered hygroscopic samples:
  more GENE*Spin* Lysis Buffer than indicated can be used until the lysate is at least semifluid and can be pipetted. Extraction can be improved by pre-incubation of sample with GENE*Spin* Lysis Buffer for 1-2 h.

If according to local regulations, defined amounts of sample have to be analyzed, higher amounts of sample (e.g. 1 or 2 g) can be used with upscaled lysis buffer volumes. We recommend to use only one 300 µL aliquot of the clear supernatant per GENE*Spin* Column. Otherwise, prepare 2 aliquots and load them step by step onto the GENE*Spin* Column.

GENE*Spin* Lysis Buffer can be ordered separately (Cat no. 5224701901)

11. TROUBLESHOOTING

Problem	Possible cause
DNA yield is low	• Grinding/homogenization of food material was not sufficient • Extraction of DNA from sample during lysis was not sufficient. • Sample contains too much RNA • Suboptimal elution
DNA is degraded	• Sample contamination with DNase • Centrifugation speed was too high
DNA purity is poor	• DNA contaminants are not fully removed

Suggestions

• For most matrices grinding with commercial bead mills/ mixers/homogenizers or steel beads is recommended.
• For higher yields of DNA, the lysis incubation time can be prolonged (up to overnight).
• Add 10-20 µL of RNase A solution to the Lysis Buffer before heat incubation. If this is not successful, add the enzyme to the cleared lysate and incubate for 30 min at 37°C.
• The DNA can be either eluted in higher volumes (up to 300 µL) or by repeating the elution step up to three times. Remember that the GENE*Spin* Elution Buffer must be preheated to 70°C.
• Check if the pH of your elution buffer is in the range of 8.0 - 8.5. Preferentially use the supplied GENE*Spin* Elution Buffer.

• Check working area and pipettes and clean, if necessary.
• Centrifuge at speed indicated in the protocol. Higher speed and prolonged vortexing can lead to shearing of the DNA.

• Repeat washing step with GENE*Spin* Wash Buffer 1.

12. SAFETY INSTRUCTIONS

The following components of the GENESpin kit contain hazardous contents.
Wear gloves and goggles and follow the safety instructions given in this section.

GHS classification

GENE*Spin* **Proteinase K:** lyophilized	H315, H319, H334, H335 **Danger** 	P261, P280, P302+P352, P304+P340 P305+P351+P338 P342+P311
GENE*Spin* **Binding Buffer** Guanidine hydrochloride 36-50 %	H302+H332 H315 H319 **Warning** 	P260, P280 P301+P312 P302+P352 P304+P340 P501

Component	H-phrases	P-phrases
GENE*Spin* **Wash Buffer 1:** Guanidine hydrochloride 24-36 %, Ethanol 35-55 %	H226, H302+H332 H315 H319 **Warning** 	P210 P241 P260 P280 P301+P312 P501

Hazard phrases

H226 Flammable liquid and vapour.
H302+H332 Harmful if swallowed or if inhaled.
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.

Precaution phrases

P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P241 Use explosion-proof electrical, ventilating, lighting equipment.
P260 Do not breathe spray, vapours.
P261 Avoid breathing dust.
P280 Wear eye protection, protective gloves, protective clothing.
P301+312 IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P302+352 IF ON SKIN: Wash with plenty of water, soap.
P304+340 IF INHALED: Remove person to fresh air and keep comfortable for breathing.

P305+351+338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P342+311 If experiencing respiratory symptoms: Call a POISON CENTER or doctor/ physician.
P501 Dispose of contents/ container to an approved waste disposal plan.

For further information see Material Safety Data Sheet.

13. PRODUCT WARRANTIES AND SATISFACTION GUARANTEE

Gold Standard Diagnostics warrants the products manufactured by it will be free of defects in materials and workmanship when used in accordance with the applicable instructions for a period equal to or shorter of one year from the date of shipment of the product(s) or the expiration date marked on the product packaging under the storage conditions, recommended in the instructions and/or on the package. Application protocols published by Gold Standard Diagnostics are intended to be only guidelines for the buyers of the products. Each buyer is expected to validate the applicability of each application protocol to his individual application. Gold Standard Diagnostics makes no other warranty, expressed or implied. There is no warranty of merchan-tability or fitness for a particular purpose.

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14. IMPORTANT NOTES

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

15. TECHNICAL SERVICE

If you have any questions or experience any difficulties regarding this kit or Gold Standard Diagnostics products in general, please do not hesitate to contact us.

Gold Standard Diagnostics customers are also a major source of information regarding advanced or specialised use of our products. This information is helpful to other scientists as well as to the researchers Gold Standard Diagnostics. We therefore encourage you to contact us if you have any suggestions concerning product performance or new applications and techniques.

For technical assistance and more information please contact the Gold Standard Diagnostics TechSupport department or your local distributor.

GENESpin
Short Protocol

Homogenize	Homogenize 200 mg material	
Lyse cells	+ 550 µL Lysis Buffer (65°C) + 10 µL Proteinase K 65°C, 30 min > 10,000 x g, 10 min	
Adjust DNA binding conditions	Clear supernatant (1 volume) + 1 vol. Binding Buffer + 1 vol. ethanol	
Bind DNA	Load 700 µL sample stepwise (max. loading capacity 750 µL) 11,000 x g, 1 min	
Wash and dry	1st wash 2nd wash 3rd wash	+ 400 µL Wash Buffer 1 11,000 x g, 1 min + 700 µL Wash Buffer 2 11,000 x g, 1 min + 200 µL Wash Buffer 2 11,000 x g, 2 min
Elute DNA	+ 100 µL Elution Buffer (70°C) RT, 5 min 11,000 x g, 1 min	

TECHNICAL SUPPORT SERVICE

For additional information and contact details please visit https://www.goldstandarddiagnostics.com/contacts.