For laboratory use only

BioKits
DNA Extraction Kit
(Speciation)

Up to 100 DNA extractions

For the extraction of DNA from foods & feeds prior to PCR analysis

Store below -18°C

Contents

1. Introduction

The identification of species content is performed in many countries for a variety of reasons, both economic and ethnic. Such identification may be designed, for example, to prevent the substitution of meat destined for human consumption with that of unsuitable or inferior species, or may be of importance in various religious communities where consumption of a particular species is proscribed. Authentication of fish species has also been difficult, particularly from cooked, mixed matrices. Polymerase Chain Reaction (PCR)[1] methodology can be used to establish the presence or absence of species-specific DNA sequences. This technique has been found to be applicable to both raw meats & fish, processed food and feedstuffs.

The extraction of DNA from food matrices presents a number of challenges unique to this field. Inhibition of PCR reactions can result if a DNA extract contains e.g. high salt, sugar or protein concentrations, or has an extreme pH value. In order to produce DNA of suitable quality for use in PCR tests, there is a need for a simple, effective DNA extraction method that can be applied to a wide range of food/feed matrices.

2. Intended Use

The BioKits DNA Extraction Kit (Speciation) is intended to be used for the purification of high quality DNA from raw and cooked meats & fish, as well as a wide range of food and feed matrices prior to PCR analysis for the identification of specific species in food and food ingredients, animal feedstuffs etc.

The magnetic beads can also be used for post-purification clean-up of problematic DNA extracts. Please contact your Distributor or Neogen for further information.

3. Principles of the method

The BioKits DNA Extraction Kit (Speciation) utilises proprietary magnetic beads with a high affinity for DNA to optimise the extraction of DNA of high purity from complex food/feed matrices.

Samples are initially rendered homogeneous, then magnetic particles are used to selectively purify DNA molecules from sample lysates. DNA in the lysate is bound by the beads and the bead/DNA complex is washed with an ethanol solution. DNA is eluted off the magnetic beads using a buffered solution.

This selective binding process eliminates the need to precipitate DNA out of solution, a step which can cause problems when extracting from complex food/feed matrices. With DNA tightly bound to magnetic beads, effective washing stages ensure that there is less chance that the extracted DNA will cause inhibition during subsequent PCR testing.

[1] The PCR Process is covered by patents owned by Hoffman La Roche Inc. Use of the PCR process requires a license. Nothing in this guide should be construed as an authorisation to perform PCR under any patents held by Hoffman La Roche.

SAMPLE PREPARATION & EXTRACTION FLOWCHART

Homogenise entire sample or at least 5 x 20 g portions

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Add Tissue Extraction Solution 1 and Proteinase K to sample
Increase volumes of Solution 1 and Proteinase K if initial volume is absorbed

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Mix sample by vortexing
Incubate at 65°C for 60 minutes with constant shaking

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Transfer 1.0 ml of lysate to clean Eppendorf tube

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Cool samples on ice for 5 minutes.
Add 54 µl Extraction Solution 2

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Invert tubes to mix. Incubate samples on ice for 10 minutes

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Centrifuge samples at >8,000 g for 5 minutes

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Remove 400 µl of supernatant and add to 50 µl of **washed** magnetic beads

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Add 400 µl DNA Binding Solution. Mix.
Incubate at RT for 5 minutes

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Wash beads x2 using 500 µl 75% ethanol.
Dry beads at 65°C for 5 minutes

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Elute DNA in 2 x 100 µl TE Buffer at 65°C

4. Kit Components

The following reagents are supplied in the kit (for up to 100 extractions):

Component	Volume	Storage Conditions
Tissue Extraction Solution 1 (Please see caution below)	600 ml	Ambient
Tissue Extraction Solution 2	5.5 ml	Ambient
DNA Binding Solution	45 ml	Ambient
Magnetic Beads	5.5 ml	Ambient
Tris-EDTA (TE) Buffer	50 ml	Ambient
Package Insert	N/A	N/A

Accessory Pack (Cat No. 903003J)		
Proteinase K Solution (10 mg/ml)	2 ml	-20°C
Agarose Gel Loading Dye	550 ml	4°C

Caution: contains 30% guanidinium hydrochloride (harmful if swallowed; irritant to eyes and skin).

Wear gloves, lab coat and safety glasses when handling

5. Materials / Equipment required but not provided

- Analar or similar grade Ethanol
- Molecular Biology Grade Water (MBGW)
- Ice or other chilling apparatus e.g. Chillette, portable cooler
- 1.5 ml 'Eppendorf' type tubes with integral locking lids
- 30 & 50ml graduated disposable polypropylene tubes e.g. Falcon
- Two place balance
- Microlitre type pipettes (sizes 20, 200, 1000 µl) and disposable filter tips
- Vortex mixer
- Magnetic rack (TBS Cat. No. 901038Y) **WARNING – STRONG MAGNETIC FIELD**
- 65°C shaking incubator
- Centrifuge for Eppendorf tubes – capable of 13,000 g
- UV spectrophotometer, capable of reading at 260 nm
- QUARTZ CRYSTAL cuvettes (1ml or 0.5 ml in size)

6. Safety / COSHH Note:

The techniques of "good laboratory practice" should be employed when using the kit. If such practices are used the reagents constitute a very low potential risk to health. Safety clothing (lab coat, glasses and gloves as necessary) should be worn and skin contact with reagents avoided; do not ingest. Any contact with skin/eyes should be treated by washing/ irrigation. It is also important to be aware of the allergic, toxic or infectious potential of analytical samples.

Safety information relating to this product is available from Neogen Europe Ltd.

7. Shelf Life

Extracted Samples: After extraction, DNA solutions may be stored for at least six months if refrigerated at 2–8 °C or for at least one year if frozen at or below -20°C.

Kit Reagents: With the exception of Proteinase K, the BioKits DNA EXTRACTION KIT (Speciation) can be stored at ambient temperature.

PROTEINASE K reagent MUST BE STORED AT -20°C. Storage above this temperature will significantly affect the efficiency of the enzyme.

The shelf life of the unopened kit is indicated on the outer label and those of its components are indicated by the expiry date on their respective labels.

Occasionally, when ongoing stability testing by Neogen indicates that shelf life can be extended, the shelf lives on these labels may be updated via a Customer Information Sheet packed in the kit.

Once the kit reagents have been opened they should be stable for at least six months (or until expiry, whichever is sooner).

8. Preparation of samples

Prepare a solution of 75:25 (v/v) ethanol : MBGW (approx. 100 mls). Excess solution can be stored at ambient temperature.

Homogeneous liquids need no preparation.

For animal feeds etc. use a blender, mill, grinder or pestle & mortar, to reduce a large sample (at least 50 g) to as small a particle size as possible to ensure homogeneity.

Safety note: Dry samples especially should be handled in a fume hood or similar apparatus to prevent breathing dust. If the hood is not of the containment type, a facemask should also be worn to prevent inhalation of dust particles. Appropriate protective clothing should be worn at all times when handling the samples and extracts.

9. Preparation of components

Tissue Extraction Solution 1

Tissue Extraction Solution 2

DNA Binding Solution

Magnetic Beads

Proteinase K

TE Buffer – ready for use

Agarose Gel Loading Dye

For all reagents, no preparation is required other than mixing contents thoroughly by repeated inversion (do not shake).

Extraction Control

1. Add 400 µl of Tissue Extraction Solution 1 to a clean Eppendorf tube. Add 10 µl Proteinase K solution. This is the extraction control and should be processed in an identical fashion to all other samples. Proceed to step 5.

Raw Meat and Fish Samples

2. Weigh out 20–40 mg of homogenised material into a 1.5 ml Eppendorf tube and add 400 µl of Tissue Extraction Solution 1. Add 200 µg Proteinase K (20 µl of 10 mg/ml solution). Proceed to step 5.

Other Samples

3. For all other samples weight 2 g into a 30 ml sample tube and add 5 ml of Tissue Extraction Solution 1 and 200 µg of Proteinase K (20 µl of 10 mg/ml solution). If the sample absorbs all added liquid, increase Tissue Extraction Solution 1 volume to 10 ml and Proteinase K to 400 µg. Further increases in volume may be performed, if necessary, to a total of 20 ml + 800 µg. Proceed to step 5.

Liquid Samples (i.e. Milks)

4. Measure out 5 mls of the sample and add 2.5 mls of Tissue Extraction 1 and 500 µg of Proteinase K (50 µg of a 10mg/ml solution). Proceed to step 5.

Sample Lysis

5. Samples must be vortexed to ensure thorough mixing and then incubated at 65°C for 60 minutes with constant shaking.

6. Transfer 1.0 ml of each sample (transfer entire 400 µl from a raw meat sample – see 2.) into a clean Eppendorf tube.

7. Steps 8–10 must be performed for mixed matrix samples, such as animal feeds. For samples such as raw meats, continue from Step 11.

8. Cool samples on ice for 5 minutes. Add 54 µl Tissue Extraction Solution 2.

> **Note:** It is important to cool samples as protein precipitation will be inefficient if sample is too warm.

9. Invert tube to mix. Ensure no lumps are present by flicking base of tube several times.

10. Place samples in ice for a further 10 minutes to facilitate protein precipitation.

11. Centrifuge samples at >8,000 g for 5 minutes to sediment any debris.

Magnetic Bead Preparation

12. During the sample centrifugation, prepare magnetic beads for use as follows:

12.1 Gently agitate Magnetic Beads to ensure beads are in suspension.

12.2 Dispense 50 µl of beads into a clean Eppendorf tube for each sample. Immobilise beads by placing tubes in the magnetic stand. Remove liquid using a pipette.

12.3 Remove the tubes from the stand and wash the beads by re-suspending them in 500 µl of MBGW.

12.4 Immobilise beads by placing tubes in the magnetic stand and remove the water using a pipette.

DNA Extraction

13. Move the Eppendorf tubes to the row of holes furthest away from the magnets and add ~ 400 µl from the sample supernatant to the magnetic beads, taking care to avoid any debris in the lysate.

14. Add an equal volume of DNA Binding Solution to the magnetic bead/sample lysate mixture. Flick the base of the tube to ensure that the beads are totally resuspended.

15. Leave samples to incubate at room temperature for 5 minutes. Ensure optimal mixing by flicking base of tube every minute.

16. Immobilise the beads by placing the Eppendorf tubes against the magnets in the magnetic stand. Invert the stand several times to wash beads from the sides of the tube.

17. Leave the Eppendorf tubes by the magnets for 2 minutes than carefully remove and discard the supernatant.

18. Move the Eppendorf tubes to the row of holes furthest away from the magnets. Wash the bead/DNA complex by resuspending in 500 µl 75% ethanol. If the magnetic bead pellet is difficult to resuspend, flick the tube and then invert 5–10 times. Immobilise the beads by placing the Eppendorf tubes against the magnets and remove all excess ethanol.

19. Repeat Step 18.

20. Incubate the samples (leaving tube lids open) for 5 minutes at 65°C or air dry at room temperature for 15 minutes to evaporate off any remaining ethanol.

Note: Do not dry beads at 65°C for longer than 5 minutes.

21. Remove and discard and residual solvent left in the base of the Eppendorf tubes using a pipette.

Note: It is important to ensure there is no solvent left in the tube prior to DNA elution.

22. Add 100 µl of TE Buffer. Flick each tube gently to resuspend the beads and incubate at 65°C for 10 minutes.

23. Ensure the beads remain in suspension by flicking the tubes gently after 5 minutes and again after the 10 minutes have elapsed.

24. Centrifuge the tubes for 5 seconds to collect any condensation.

25. Immobilise the beads in the magnetic rack and pipette the supernatant into a second clean Eppendorf tube. This solution contains the purified genomic DNA.

26. Repeat Steps 22–25 using a further 100 µl of TE Buffer. Carefully remove the supernatant and combine the two eluates in the second tube.

27. To ensure the final eluted sample is bead free, place the combined eluate against the magnets in the magnetic stand and leave for 2 minutes. Any remaining beads will be observed as a small black pellet in the base of the tube near the magnet.

28. If a pellet of magnetic beads is visible, remove the supernatant to another clean Eppendorf tube.

29. Store DNA extracts at 4°C. If long term storage is required (more than 6 months) the sample should be divided into 5 µl aliquots and stored at -20°C.

11. Analysis of yield

1. In order to quantify the amount of DNA extracted from each sample, a spectrophotometric reading of a dilution of the extract can be taken.

2. QUARTZ CRYSTAL CUVETTES MUST BE USED FOR THE MEASUREMENT

3. For each sample, a 1 in 25 dilution of the DNA extract should be prepared in MBGW. If using a 1 ml cuvette, 40 µl of DNA should be added to 960 µl of MBGW. For a 0.5 ml cuvette, 20 µl of DNA should be added to 480 µl of MBGW. All dilutions must be thoroughly mixed by vortexing prior to analysis.

4. The UV spectrophotometer must be set to read at 260 nm. Blank the spectrophotometer using a cuvette containing MBGW before analysing any sample DNA.

5. A solution containing 50 µg/ml of genomic DNA will give an A260 reading of 1.0. This figure is used in the following calculation to determine the concentration of DNA in the original solution:

For example, a sample with an A260 reading of 0.426 has a concentration of:

0.426 x 25 x 50 = **532.5 ng/µl** (532.5 µg/ml)

where '25' is the dilution factor (as in Step 11.3), and '50' adjusts the OD reading for the DNA concentration.

6. Absorbance readings should ideally be above 0.04.
7. When diluting DNA extracts for PCR, a total of between 100 and 200ng of DNA should be tested per reaction i.e. 5 µl of a 20 ng/µl DNA solution.

Note: Certain samples may produce a coloured DNA solution when eluted. Any colouration in the final DNA extract may result in an artificially high A260 reading.

Any cloudy DNA extracts must be centrifuged (5–10 minutes at 13,000 rpm) prior to taking an aliquot for spectrophotometric analysis.

12. Performance Characteristics

In our laboratories the above extraction procedure has been used successfully to purify DNA from a wide variety of food samples. Samples tested include e.g.:

Raw and processed meats & fish

Cheeses

Milks

Animal feeds

Ready meals

Vegetarian products

It should also be noted that successful speciation can be achieved even though there is no detectable DNA (section 11 above).

General Notes:

Ensure that tubes are labelled consistently throughout the extraction procedure, as solutions are transferred from sample lysate, to magnetic beads to final storage.

If extracting from a large number of absorbent samples (i.e. animal feeds), the amount of Tissue Extraction Solution 1 supplied may be limiting. Please contact your Distributor or Neogen Europe Ltd. for further supply of reagents.

For samples where low yields of DNA may be expected (e.g. highly processed samples, such as corned beef), elution can be performed using 2 x 50 µl volumes rather than 2 x 100 µl volumes.

For agarose gel analysis of extracted DNA, add approximately 3 µl of the agarose gel loading dye provided, to 10 µl of DNA solution.

13. Troubleshooting

What if the sample is dry after incubation at 65°C?

Samples should be checked after 15 minutes of incubation time to ensure that this situation is avoided. More extraction solutions can be added, and a further 15 minutes incubation carried out if samples are found to absorb all liquid.

Is the extraction protocol the same for all samples, raw and cooked?

Yes. The standard protocol can be used for both raw and cooked samples. There are slightly modified procedures available for extraction of DNA from animal feeds, gelatin and liquid samples.

What if the sample supernatant is still cloudy after centrifugation?

The supernatant can be removed from the tube and re-centrifuged at a higher speed (>10,000g) in order to try and pellet any remaining debris.

Why is the eluted DNA solution yellow?

Coloration of the DNA solution can be caused by incubating the beads for longer than the designated time when evaporating off solvent at 65°C. This can easily be avoided by drying the beads for only 5 minutes and no longer. Any solvent remaining after this time can be removed using a pipette. Occasionally, coloured sample lysates can also cause eluted DNA solutions to be tinted; these are usually still viable for PCR. When performing a PCR assay with a tinted DNA eluate, a spiked reaction should always be run to ensure no PCR inhibitors are present.

What if the eluted DNA solution is cloudy?

This situation can occur if protein precipitation has been inefficient, or if there is a high concentration of starch in the sample. Cloudiness is sometimes only apparent after samples have been stored overnight at 4°C. Cloudy DNA solutions should be centrifuged at approximately 10,000g. The clear supernatant containing the DNA can then be removed to a fresh tube.

Why does the DNA seem to float out of the well when loading the agarose gel?

This is caused by ethanol being present in the final eluate. Care must be taken to remove all solvent from the beads after the ethanol wash steps.

Will increasing the final elution volume result in a greater yield of DNA?

No. Two elutions are performed which ensures that all of the DNA has been removed from the beads. Increasing the elution volume will only result in your final DNA eluate being less concentrated.

If you would like further assistance with this product, contact Neogen Europe Ltd. or your local distributor and, if possible, provide the following information:

- Kit type, Catalogue and Batch numbers.
- Brief details of the samples being tested and preparation/extraction methods.
- Equipment used.

This ensures that Neogen Europe Ltd. can respond promptly and fully to your needs.

Related Products:

Description (Extraction Kit not included)

Animal Speciation PCR Selection Module, choice of 2 or 3 PCR Pods from:	**Catalogue No.**
Chicken	503096D
Turkey	503097B
Beef	503094H
Goat	503099W
Pork	503093K
Horse	503098Y
Sheep	503095F
Rabbit	503100R

Fish Speciation PCR Selection Module, choice of 2 or 3 PCR Pods from:	**Catalogue No.**
Cod	503130G
Coley	503132C
Hake	503131E
Haddock	503133Z
Pollock	503134X
Whiting	503135V
Atlantic Salmon	503137R
Rainbow Trout	503136T

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