



BIOTOOLS

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BIOFOOD Kits

***Kits for vertebrate species detection and identification in
food using genetic markers***

(Cat. No. 91.122)

IDENTIFICATION KIT

Package Insert

PLEASE READ THE INSTRUCTIONS FOR USE THOROUGHLY BEFORE USING THE KIT, ESPECIALLY IF YOU ARE NOT FAMILIAR WITH THE PROTOCOL.

GENERAL INFORMATION

BIOFOOD kits allow the detection and, depending on the specific kit, the precise identification of a high number of animal species in food and feed samples, fresh and processed. The detection method is based on the stability of nucleic acids, that endure the processes used in food industry (temperature, vacuum, drying, etc.). The kit has been tested with fresh and highly processed samples (including animal feed). DNA is purified from the samples, in order to be amplified and analysed by agarose gel electrophoresis.

BIOFOOD IDENTIFICATION detects the presence of vertebrate material (mammal, reptile, bird, amphibian or fish¹) in a sample by means of exponential amplification of a mitochondrial gene which is highly conserved throughout evolution (cytochrome b). This gene has been chosen due to its high cell copy number, as well as availability of sequences for a high number of species in databases. Amplified product is submitted to restriction analysis (RFLP), so that the species present in the sample can be **identified**. This is possible due to minute differences in the sequence of the amplified fragment, so that restriction patterns are species-specific.

Unless otherwise indicated the Kit includes control DNAs from pork, turkey, chicken and goat. For other possible DNA controls contact our Technical Dept. (info@biotools.eu).

RESEARCH USE ONLY

Some of the applications which may be performed with this product may be covered by applicable patents in certain countries. The purchase of this product does not include or provide a license to perform patented applications. Users may be required to obtain a license depending on the country and/or application. Biotools does not encourage the unlicensed use of patented applications.

PLEASE CHECK KIT AND REAGENTS INTEGRITY BEFORE USE. USE OF DETERIORATED KITS MAY CAUSE LACK OF RESULTS AND/OR EQUIVOCAL RESULTS.

PRINCIPLE

DNA is obtained either from fresh or processed samples using an optimised method² (we recommend using Genomic DNA Extraction Kit - Cat. No. 21.002, 24 tests, or 21.003, 48 tests, available in our catalogue). Use of other methods is possible. However, user must confirm that the purified DNA can be used with the kit (concentration 50-100 ng / μ l, $A_{260/280}=1.8 - 2.0$, absence of inhibitors that may affect the result of the amplification reaction, etc.). It is recommended to check the quality and suitability of the purified DNA for amplification reactions, e.g., performing control amplifications in parallel.

BIOFOOD IDENTIFICATION kit is based on the detection and amplification of cytochrome b from vertebrates, followed by restriction analysis in order to obtain species-specific restriction patterns, which are analysed by agarose gel electrophoresis.

BIOFOOD IDENTIFICATION kit can be used with heterogeneous samples (more than one component). However, presence of 3-5 species in a sample may cause difficulties in interpreting the restriction patterns. Sensitivity of the kit ranges between 0.5 – 5 %, depending on the identity of the species present in the sample and their relative percentage.

REAGENTS

The Kit contains reagents in liquid format for performance of 48 amplification reactions (Cat. No. 91.122). To minimize the risk of contamination and facilitate the use of the Kit several times the Kit is presented in two set of 24 reactions. Sample Kit (Cat. No. 91.121) contains one set of 24 reactions. Thaw and handle reagents on ice. **Do not freeze/thaw Kit vials repeatedly.** In case of frequent use, we **recommend the aliquoting of the vial contents.**

- **Master Mix** Two vials: 2 x 405 μ l
A Tris-HCl solution, containing <10 % glycerol, KCl, <0.001 % dATP, dCTP, dGTP, dTTP and primers. Master Mix includes all amplification reagents for the detection of the cytochrome b, except $MgCl_2$ and DNA polymerase, in the adequate ratios.
Store at **-15 \pm 8 °C**.
- **MgCl₂ Solution (50mM)** Two vials: 2 x 1.8 ml
Store at **-15 \pm 8 °C**. Mix well before use.
- **DNA Polymerase (1U/ μ l)** Two vials: 2 x 50 μ l
Store at **-15 \pm 8 °C**. Add to reaction mixture shortly before introduction of vials in thermal cycler
- **Enzyme 1** Two vials: 2 x 14 μ l
Store at **-15 \pm 8 °C**.

¹ Detection is for vertebrate in general, without determining what group of vertebrate is present.

² Food and feed samples, due to their composition (additives, colourings, preservatives) have a high amount of components that may inhibit amplification reactions. Therefore, it is a must that the DNA purification method eliminate these inhibitors, keeping DNA integrity.

- **Enzyme 1 Buffer** Two vials: 2 x 54 µl
10X buffer for Enzyme 1
.Store at **-15±8 °C**.
- **Enzyme 2** Two vials: 2 x 14 µl
Store at **-15±8 °C**.
- **Enzyme 2 Buffer** Two vials: 2 x 54 µl
10X buffer for Enzyme 2
Store at **-15±8 °C**.
- **Enzyme 3** Two vials: 2 x 14 µl
Store at **-15±8 °C**.
- **Enzyme 3 Buffer** Two vials: 2 x 54 µl
10X buffer for Enzyme 3
Store at **-15±8 °C**.
- Amplification Controls (Positive Controls)
DNA amplification products at 10⁶ copies/µl from 4 different origins. Unless otherwise indicated the kit included controls are:

Pork Amplification Control	Two vials: 2 x 40 µl
Turkey Amplification control	Two vials: 2 x 40 µl
Chicken Amplification Control	Two vials: 2 x 40 µl
Goat Amplification Control	Two vials: 2 x 40 µl

Store at **-15±8 °C**. Thaw and handle on ice. Do not freeze/thaw repeatedly. For frequent use, we recommend the aliquoting of the vial contents.

Available control DNAs in Biotools' catalogue are^{3*}:

- | | | |
|---------|--------|----------|
| * Cow | * Dog | * Sheep |
| * Horse | * Cat | * Rabbit |
| * Human | * Duck | * Goose |

MATERIALS REQUIRED BUT NOT PROVIDED

NOTE

For all equipments, regular maintenance and calibration is necessary. Follow manufacturer's instructions, and check working parameters regularly, specially for thermal cyclers and pipettes. Maintenance and calibration of instruments allows its correct functioning, and helps detecting problems that may render an incorrect analysis result.

Pre-amplification area

- Equipment, reagents and disposable material necessary for DNA purification (depending on the method, follow manufacturer's instructions)
- Timer
- Automatic pipettes⁴ (10, 20 and 200 µl), filter or positive displacement tips, RNase-free⁵
- Disposable examination gloves, powder-free
- Sterile bidistilled water
- Screw cap polypropylene tubes, 1.5 ml capacity, non siliconised, conical, sterile, RNase-free. It is recommended to use screw cap tubes, in order to avoid the potential contamination of samples and controls.
- Racks for 1.5 ml vials
- Containers for disposal of potentially-infectious material
- Disposable filter paper for working surface, cleaning paper for accidental spills
- Termi-DNA-Toi⁶ or equivalent, in order to remove DNA from working surfaces

³ The list of available control DNAs is continuously growing. Should you be interested in a particular bird or mammalian species not included in this list, please do not hesitate to contact us for further information.

⁴ Precision of automatic pipettes must be in the range of 3 % of the indicated volume. If necessary, calibrate and check regularly, following manufacturer's instructions. It is recommended to use RNase-free filter tips and positive displacement tips, in order to avoid cross contamination between samples and amplicons.

⁵ It is recommended to use different sets of pipettes for each reaction step (pre-amplification, amplification, post-amplification), in order to avoid contaminations that may render false positive results.

⁶ Available in Biotools' catalogue (Cat. No. 40.201).

Amplification area

- Thermal cycler: Eppendorf MasterCycler™ Personal, MJ Research MiniCycler™ or Applied Biosystems GeneAmp™ 2700. Use of this kit in other equipments has not been tested. For further information, contact our Technical Dept. (info@biotools.eu).
- Laminar flow cabinet
- Racks for reaction vials
- Reaction vials (0.2 ml, thin-walled)
- Sterile bidistilled water (Cat. No. 20.033 or equivalent)
- Automatic pipettes (10, 20 and 200 µl), filter or positive displacement tips, RNase-free
- Disposable examination gloves, powder-free
- Containers for disposal of potentially-infectious material
- Disposable filter paper for working surface, cleaning paper for accidental spills
- Termi-DNA-Tor or equivalent, in order to remove DNA from working surfaces

Post-amplification area

- Electrophoresis power supplies and tanks
- Gel Documentation system
- UV transilluminator
- Ethidium bromide
- Low EEO agarose (Cat. No. 20.011) or equivalent
- TAE or TBE
- DNA Ladder ranging between 150 to 700 bp (Cat. No. 31.006) or equivalent
- Electrophoresis loading buffer
- Automatic pipettes (10, 20 and 200 µl), filter or positive displacement tips, RNase-free
- Disposable examination gloves, powder-free
- Protective mask / goggles for UV
- Microwave

PROTOCOL

NOTE

Thaw all reagents on ice. Keep on ice while in use. Amplification must be started in the next 10 minutes after adding the purified DNA and controls to the amplification mix. Check thermal cycler regularly. Non-existent or poor calibration of the equipment may render equivocal results. Este protocolo ha sido adaptado para equipos de amplificación Eppendorf, MJ Research y Applied Biosystems GeneAmp™.

This protocol has been adapted for Eppendorf, MJ Research and Applied Biosystems GeneAmp™ thermal cyclers. For other thermal cyclers, optimisation of reaction parameters may be necessary. For any question, please contact our Technical Dept. (info@biotools.eu).

1.- Final reaction volume is 50 µl. Calculate the necessary volume of the **Master Mix**, **MgCl₂**, **DNA Polymerase** and **Positive Control** for the analysis of samples and controls. It is recommended to perform one Positive Control and one Negative Control in each round of analysis (this must be taken into account when calculating necessary volume for performance of all reactions).

2.- Mix the necessary volume of **Master Mix**, **MgCl₂** and **DNA Polymerase** for the number of reactions to perform in a 1.5 ml vial. **Perform this process in a laminar flow cabinet.** Keep the reaction mixture (reaction mixture = Master Mix + MgCl₂ + DNA Polymerase + sterile bidistilled water) on ice:

Reagent	For 3 reactions	For 10 reactions	For n reactions
Master Mix	45 µl	150 µl	15 * n µl
MgCl ₂	6 µl	20 µl	2 * n µl
DNA Polymerase	3 µl	10 µl	1 * n µl

3.- Aliquot 40 µl of the reaction mixture in each amplification vial, **in the laminar flow cabinet.**

4.- Remove vials from laminar flow cabinet. Add 50-100 ng from DNA from the purified samples and/or controls to each amplification vial. Complete up to 50 µl final volume.

NOTE

For control reactions, use 5 µl of Positive Control (except cat and dog, where 20 µl should be added). For negative controls, use 5 µl of sterile bidistilled water.

5.- Close amplification vials. Place them in thermal cycler. Store remaining of all reagents at temperatures under -18°C .

Perform the amplification according to the following program:

94 °C	1 min 30 sec		
94 °C	10 sec	}	35 cycles
55 °C	30 sec		
72 °C	40 sec		
72 °C	3 min		
4 °C	∞		

6.- After amplification, result can be checked by 1 % agarose gel electrophoresis. A 359 bp band must be obtained for samples positive to vertebrate material. Store amplification product at $2-8^{\circ}\text{C}$, unless restriction analysis is to be performed in the next 1-2 hours.

7.- Transfer 10 μl of amplification product to a new vial, and add 7.5 μl of sterile bidistilled water.

8.- Add 2 μl of the corresponding 10X buffer (1, 2 or 3)

9.- Add 0.5 μl of the corresponding restriction enzyme (1, 2 or 3)

10.- Incubate 2 hours at 37°C . For optimal results, incubate for 3 hours.

INTERPRETATION OF RESULTS

The analysis of amplification products is performed by horizontal electrophoresis in low EEO-agarose gels (e.g. MB Agarose, Cat. No. 20.011). Band visualisation is improved in 1 % gels using TAE 1X or TBE 0.5X as running buffers. It is recommended to add ethidium bromide in the agarose gel for a better resolution and visualisation. For restriction pattern analysis, use 2-3 % gels using TAE 1X or TBE 0.5X as running buffers. It is recommended to add ethidium bromide in the agarose gel for a better resolution and visualisation.

NOTE

Ethidium bromide is a highly mutagenic intercalating agent. Use of gloves and maximum caution is recommended on handling this reagent.

Samples containing vertebrate sequences will render a band of 359 bp. Compare RFLP patterns with the table as well as Figure 1.

Species	Enzyme 1	Enzyme 2	Enzyme 3
Cow	286 73	359	198 117 44
Pork	153 127 73 6	359	359
Chicken	159 127 73	211 148	188 161 10
Turkey	127 103 73 56	148 110 101	198 161
Sheep	159 127 73	359	296 63
Goat	230 73 56	359	198 161
Cat	253 73 22 11	213 122 24	117 (x2) 81 44

Species	Enzyme 1	Enzyme 2	Enzyme 3
Duck*	286 73	--	359
Horse	159 105 73 22	359	234 81 44
Dog	232 127	283 42 34	296 54 9
Rabbit	153 133 43 30	359	233 126
Human	232 105 22	359	198 161
Goose*	153 133 73	--	359

* Restriction patterns for these species have been deducted from agarose gels, as there is no accurate information in databases. Pattern corresponding to Enzyme 2 has not been clearly assigned.

NOTE

Due to sequence differences between samples of different brew or geographical origin, some restriction patterns may vary from those indicated in the table. Please contact our Technical Dept. for further information.

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 M

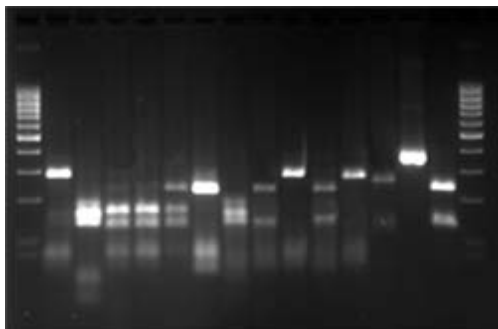


Figure 1: Visualisation of RFLP fragments belonging to 14 different species after digestion with restriction endonuclease 1 included in this kit. M: 100-bp molecular ladder (Cat. No. 31.006). Lanes 1 to 14: restriction profiles from water buffalo, rabbit, horse, roe deer, cow, goat, deer, emu, red kangaroo, ostrich, cat, dog, mouse and human.

QUALITY CONTROL

It is recommended that at least one (1) Positive Control and one (1) Negative Control be run each time the test is performed. As with any new laboratory procedure, novel users should consider performing additional controls (both positive and negative) until a high degree of confidence is reached.

The Positive Control must render the corresponding bands (see 'Interpretation of Results'). Vials containing negative control (sterile bidistilled water) must render no bands. Any analysis not fulfilling any of these results must be completely invalidated and discarded. It is necessary to repeat the process from its beginning, including DNA purification, processing other aliquot of the original sample. A failure of instruments during the test, indicated by error messages, also means that the test has not been valid. Repeat all the procedure for each sample from the amplification step.

PROCEDURAL PRECAUTIONS

1. Laboratory workflow must be unidirectional, from pre-amplification area to post-amplification area. Pre-amplification tasks must be initiated with the preparation of the reagents and sample purification. Equipments, materials and reagents must be dedicated and they must not be used for other activities or be transferred from one to another area. Gloves must be worn in each area, and must be discarded before proceeding to the next area. Equipments and materials used for setting-up of reactions must not be used for other activities, or for pipetting or processing amplified DNA or other DNA sources.
2. As with any analytical procedure, it is fundamental to use a good laboratory practice to obtain good results with this technique. Due to the high analytical sensitivity of the test, extreme care must be taken in order to keep the purity of all kit reagents and all reaction mixes. All reagents must be carefully checked in order to ascertain their purity. Discard all suspect reagents.
3. Instructions must be followed in order to obtain correct results. Should the user have any questions, please contact our Technical Dept. (info@biotools.eu)
4. This test has been validated for use with the reagents provided by the kit. The use of other amplification methods, or the use of equipment not fulfilling the specifications, may render equivocal results. User is responsible for validating the modifications for this test, in any of the indicated parameters.
5. Use powder-free examination gloves while handling reagents or samples, as well as lab coat. Wash hands thoroughly after performing the test.
6. Open and close reagent vials carefully. Observe temperature and light exposure instructions. After use, close vials and store at indicated temperatures.
7. Do not use product after expiry or best before date.
8. Kit components have been tested as a whole. **Do not interchange components** with other kits, or components from different lots
9. Nucleic acids are very sensitive to degradation by nucleases. Nucleases are present in human skin and surfaces that have been in contact with humans. Wash with Termi-DNA-Tor and cover working surfaces with suitable paper. Use powder-free examination gloves throughout the whole process
10. Extreme care must be taken when aliquoting the different volumes in each reaction step. Mix well after addition of each reagent, unless otherwise noted. Read instructions for use of automatic pipettes
11. Do not pipette by mouth

12. Packaging material included with the kit is resistant to the indicated storage conditions. Storage at different conditions can cause breakage of the material, and possible contamination of kit contents
13. Plastic material included with the kit is resistant in the normal conditions of use. Use of plastic material in extreme conditions may cause its breakage, and therefore, impossibility to use the kit
14. False negative results may be obtained due to polymerase inhibition. It is recommended to perform control reactions to distinguish between inhibition and true negatives.
15. Cross contamination between samples and exogenous DNA can only be avoided by following good laboratory practice. Instructions in this document must be strictly followed.
16. Use of this product is limited to qualified professional personnel, experienced in DNA purification and DNA amplification techniques.
17. It is important to pipet the indicated amounts, and mix well after each reagent addition. Check pipettes regularly.

WARRANTY

Products are guaranteed to conform to the quality and content indicated on each vial and external labels during their shelf life. BIOTOOLS obligation and purchaser's rights under this warranty are limited to the replacement by BIOTOOLS of any product that is shown defective in fabrication, and that must be returned to BIOTOOLS, freight prepaid, or at BIOTOOLS' option, replacement of the purchasing price.

Any complaint on damaged goods during transport must be directed to the handling or transport agent.

Product for Research Use Only. This product must be used by qualified professionals only. It is the responsibility of the user to ascertain that a given product is adequate for a given application. Any product not fulfilling the specifications included in the product sheet will be replaced. This warranty limits our responsibility to the replacement of the product. No other warranties, of any kind, express or implied, including, without limitation, implicit warranties of commercialisation ability or adequacy for a given purpose, are provided by BIOTOOLS. BIOTOOLS will not be held responsible for any direct, indirect, consequential or incidental damage resulting of the use, misuses, results of the use or inability to use any product.

Manufactured by:

BIOTOOLS, Biotechnological & Medical Laboratories, S.A. has been evaluated and certified to accomplish ISO 9001:2000 requirements for the following activities: Research and development of biotechnology products and manufacture of biotechnology and in vitro products.
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