BIOTOOLS B&M LABS.S.A.

Manufactured by:

BIOTOOLS B&M Labs, S.A.
Valle de Tobalina - 52 - Nave 39
28021 Madrid
Spain

Tel. 91 710 00 74
Fax 91 505 31 18
e-mail: info@biotools.eu
www.biotools.eu

BIOFOOD Kits

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

(Ref. 91.132)

MIXED KIT

Instructions for Use

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 identification of animal species in food and feed samples. The detection method is based on the amplification of nucleic acids by PCR. The kit has been tested with fresh and highly processed samples (including animal feed). Template DNA is extracted and purified from the samples, in order to be amplified by PCR and analysed by agarose gel electrophoresis.

BIOFOOD MIXED detects and identifies six animal species commonly used in human feed (cow, pork, chicken, horse, goat and sheep) by means of amplification of a highly conserved mitochondrial gene (cytochrome b). The selection of this cytochrome b is based on it is a gene with a high copy number in the genome of target animal species as well as because it has tiny differences between species. BIOFOOD MIXED is based on a multiplex PCR that includes a specific size band for each of detected species.

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 method. User should confirm that the purified DNA can be used as template of this kit (concentration 50-100 ng / µl, A260/280=1.8 – 2.0, and absence of PCR inhibitors). It is recommended to check the quality and suitability of the purified DNA by performing parallel to positive controls.

BIOFOOD MIXED kit is based on the detection and amplification of a cytochrome b region by several primer pairs (multiplex amplification). The kit enables to identify animal species by a specific size amplicon for each specie. The kit can not be used with heterogeneous samples (more than one component). Sensitivity of the kit: ≥ 1 %.

REAGENTS

The Kit contains enough reagents for 48 reactions (Ref. 91.132). If the kit is to be used frequently, prepare aliquots of reagents to avoid frequent freeze/thaw cycles.

- **Master Mix**
  Master Mix includes all the reagents for amplification reactions, except $MgCl_2$ and DNA polymerase, in the adequate ratios.
  Store at **–15±8 ºC**.

- **$MgCl_2$ Solution** (50mM)
  Store at **–15±8 ºC**. Mix well before use.

- **DNA Polymerase** (1U**/**µl)
  Store at **–15±8 ºC**. Add to reaction mixtures shortly prior to placing vials in the thermal cycler.

- **Amplification Controls** (Positive Controls)
  Fragments of specific DNAs prepared by PCR. BIOFOOD MIXED is provided with the following DNA controls:
  Pork Amplification control
  Horse Amplification control
  Chicken Amplification control
  Goat Amplification control
  Cow Amplification control
  Sheep Amplification control
  Store at **–15±8 ºC**

MATERIALS REQUIRED BUT NOT PROVIDED

Pre-amplification area

- Equipment, reagents and disposable material necessary for DNA purification (follow manufacturer's instructions)
- Timer
- Automatic pipettes[1] (10, 20 and 200 µl), filter or positive displacement tips, RNase-free[2]
- 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-Tor[3] or equivalent, in order to remove DNA from working surfaces

Amplification area

- Thermal cycler
- Laminar flow cabinet
- Racks for reaction vials
- Reaction vials (0.2 ml, thin-walled)
- Sterile bidistilled water (Ref. 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 (Ref. 20.011) or equivalent
- TAE or TBE
- DNA Ladder ranging between 150 to 700 bp (Ref. 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

[1] 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.
[2] 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.
[3] Available in the Biotools' catalogue (Ref. 40.201).

PROTOCOL

***NOTE:** Thaw all reagents on ice and keep on ice during their manipulation. 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.*

1.- Final reaction volume is **50 µl**. Calculate the necessary volume of the **Master Mix, $MgCl_2$**, **DNA Polymerase** and **Positive Control** for the analysis of samples and controls. It is recommended to perform one Positive Control and one Negative Control (without template) in each assay (this must be taken into account when calculating necessary volume for performance of all reactions).

2.- Prepare reaction mixture according to Table 1. **Perform this process in a laminar flow cabinet**. Keep the reaction mixture on ice:

Table 1

Reagent	**µL/ rxn**
Master Mix	42 µL
$MgCl_2$ Solution	2 µL
DNA Polymerase	1 µL

3.- Aliquot **45 µL** of the reaction mixture in each vial, in the laminar flow cabinet.

4.- Remove vials from laminar flow cabinet. And add:

- ✓ ***Positive Controls***: Add **5 µL** of specific **positive control** you want to test (provided with the kit).
- ✓ ***Samples***: Add **100 ng of purified DNA** and complete up to 50 µL with sterile bidistilled water**.**
- ✓ ***Negative control** (without template)*: Add **5 µL** of sterile bidistilled water**.**

5.- Close amplification vials, mix well and centrifuge them. Place vials in the thermal cycler.

6.- Perform the amplification according to the following program:

94 ºC	90 sec	
94 ºC	10 sec	35 cycles
55 ºC	**90 sec**	
72 ºC	40 sec	
72 ºC	10 min	
4 ºC	∞	

INTERPRETATION OF RESULTS

The analysis of amplification products is performed by horizontal electrophoresis in low EEO-agarose gels (e.g. MB Agarose, Ref. 20.011). Band visualisation is improved in 1.5-2 % 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.*

Results for positive samples are as follows:

Species	Product size
Goat	157 bp
Chicken	227 bp
Beef	274 bp
Sheep	329 bp
Pork	398 bp
Horse	439 bp

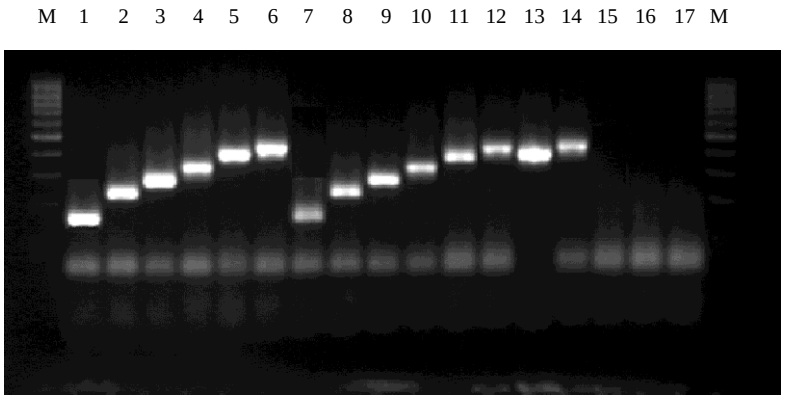

Figure 1. Species-specific amplicons: goat (lanes 1 and 7), chicken (2 and 8), cow (3 and 9), sheep (4 and 10), pork (5, 11 and 13), and horse (6, 12 and 14). DNA extractions were performed from fresh (lanes 13-14) and processed (lanes 1-12) samples, either cooked at 100ºC (lanes 1-6) or at 120ºC for 30 min (lanes 7-12). 15-16: negative control (without DNA). 17: extraction negative control. M: 100 bp DNA Ladder (Ref. 31.006).

QUALITY CONTROL

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 Dpt. (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 9001:2008 requirements for the following activities: Research and development of biotechnology products and manufacture of biotechnology and in vitro products.
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