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BIOGENICS Kits

Kits for detection of GMOs in food and food materials

Bt-11 MAIZE IDENTIFICATION KIT Cat. No. 91.234

Instructions for Use

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

Bt-11 MAIZE IDENTIFICATION KIT

For research use only

Some of the applications which may be performed with this product may be in certain countries under an applicable patent. 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. Biotoools does not encourage the unlicensed use of patented applications

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

1. GENERAL INFORMATION

BIOGENICS kits allow the detection of GMOs (Genetically Modified Organisms, transgenics) in fresh and processed samples for human and animal use. 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 (seeds, leaves, fruits, roots, flour, cookies, canned food, lyophilised, texturised, among others). DNA is purified from the samples, in order to be amplified and analysed by agarose gel electrophoresis.

BIOGENICS kits are based on the methods from the Environment Institute, Consumer Protection & Food Unit (EUR 18684 EN, Annex II), using optimised primers in order to detect the maximum number of GMOs. Sensitivity is 0.1 % minimum (though this value can be lower for some samples, depending on their composition and processing grade). This limit is under the UE threshold (1829/2003 and 1830/2003).

2. PRINCIPLE

For purification of DNA from fresh or processed samples¹ **Genomic DNA Extraction Kit (Cat. No. 21.002/3) is recommended.** The use of other methods is possible, but user must confirm that the purified DNA can be employed with the kit (concentration 50-100 ng / µl, $A_{260/280}=1.8 - 2.0$, absence of inhibitors that may affect the result of the amplification reaction, etc.). It is most important checking the quality and suitability of the purified DNA for amplification reactions, e.g. performing control amplifications in parallel.

BIOGENICS Bt-11 MAIZE kit is based on the detection and amplification of gene regions specific for Bt-11 maize (*cry1Ab* gene from *Bacillus thuringiensis* and *pat* gene from *Streptomyces viridochromogenes*) and not present in native maize, as well as GMO generic sequences (35S promoter²), present in approximately 90 % of the GMOs that have been commercialised up to date. The kit also detects the presence of generic maize (invertase gene present both in native and GMO maize) and includes control amplifications, so that discrimination between real negatives and false negatives due to amplification inhibition is achieved.

BIOGENICS Bt-11 MAIZE kit can be used with heterogeneous samples (more than one component), and detects presence of Bt-11 maize, as well as GMOs containing 35S promoter. However, it does not detect GMOs containing only NOS terminator.

3. REAGENTS

The Kit contains amplification reagents in liquid format for performance of 48 amplification reactions (Cat. No. 91.234). 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. 91.233) contains one set of 24 reactions.

- Master Mixes:

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 corresponding gene, except MgCl₂ and DNA polymerase, in the adequate ratios.

- **35S Master Mix:** for the 35S promoter identification
which indicates GMO presence. Two vials: 2 x 405 µl
- **Maize Master Mix:** for maize identification
which indicates maize presence – invertase gene, present both in native and GMO maize – Two vials: 2 x 405 µl
- **BT-11 Master Mix:** for : Bt-11 maize identification
which indicates Bt-11 maize presence – pat gene – Two vials: 2 x 405 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.

¹ 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.

² Cauliflower mosaic virus sequences (35S promoter) may be present in native plants from the *Cruciferae* family infected by this virus. Analysis of these samples should include a second control to ensure that 35S promoter presence is due to genetic manipulation.

- **MgCl₂ Solution** (50mM) Two vials: 2 x 1.8 ml
Store at –15±8 °C. Thaw on ice. Mix well before use.
 - **DNA Polymerase** (1U/μl) Two vials: 2 x 140 μl
Store at –15±8 °C. Add to reaction mixtures shortly before introduction of vials in thermal cycler.
 - Control DNAs
 - **Maize Amplification Control:** Two vials: 2 x 120 μl
 Maize control (positive to *invertase* gene). DNA amplification product containing a DNA sequence from maize *invertase* gene (10⁶ copies /μl).
 - **BT-11 Amplification Control:** Two vials: 2 x 120 μl
 Bt-11 maize control (positive to 35S promoter and *pat* gene). DNA amplification products containing DNA sequences from *pat* gene (10⁵ copies /μl) and 35S promoter (10⁶ copies /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.

4. 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 pipettes³ (10, 20 and 200 μl), filter or positive displacement tips, RNase-free⁴
- Disposable examination gloves, powder-free
- Sterile bidistilled water (Cat. No. 20.033) or equivalent
- 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 (Cat. No. 40.201/2) or equivalent, in order to remove DNA from working surfaces

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
- 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

³ 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.

⁴ It is recommended to use different sets of pipettes for each reaction step (pre-amplification, amplification), in order to avoid contaminations that may render false positive results.

Post-amplification area

- Electrophoresis power supplies and tanks
- Gel Documentation system
- UV transilluminator
- Ethidium bromide
- Low EEO agarose (Cat. No. 20.011/2) 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

5. PROTOCOL

NOTE

Thaw all reagents on ice and keep them on ice while in use.

Proceed to Pre-amplification Area in a laminar flow cabinet

1.- Final reaction volume is 50 µl (Amplification Reaction Mixture + purified DNA). Prepare each **Amplification Reaction Mixture (35S, Maize, Bt-11 pat gene)** following the table bellow (Table 1) in separates 1.5 ml vial, according to the number of reactions to be performed. For each set of amplifications reactions include at least one positive control and one negative control. To ensure sufficient volume for all reactions, prepare the Amplification Reaction Mixture for n+1 reactions.

Table 1. Preparation of Amplification Reaction Mixtures (35S, Maize, Bt-11 pat gene)

Number of reactions for each amplification mixture = number of samples + 1 positive control + 1 negative control + 1 additional

AMPLIFICATION REACTION MIXTURE FOR 1 REACTION			
REAGENT	35S	Maize	Bt-11 pat gene
MgCl ₂ Solution	2.5 µl	2 µl	2 µl
Master Mix	15 µl	15 µl	15 µl
Polymerase	1.4 µl	1.2 µl	1.6 µl
Sterile Bidistilled H ₂ O	21.1 µl	21.8 µl	21.4 µl

2.- Mix the necessary volume of all reagents and keep amplification reaction mixtures on ice.

3.- Aliquot 40 µl of the amplification reaction mixture in each amplification vial.

Proceed to DNA purification zone in the Pre-amplification Area separate from other sources of DNA (never introduce DNA in the laminar flow cabinet from the reagent preparation area). Amplification must start in the next 10 minutes after adding purified DNA from samples and controls to the amplification reaction mixture.

4.- Add 50-100 ng of purified DNA from samples to each amplification vial. Complete up to 50 µl final reaction volume with sterile bidistilled water.

5.- **Maize Positive Control** should be prepared by adding 10 µl of Maize Amplification Control vial + 40 µl of maize amplification reaction mixture. **35S Positive Control** should be prepared by adding 10 µl of BT-11 Amplification Control + 40 µl of 35S amplification reaction mixture. **Bt-11 pat gene Positive Control** should be prepared by adding 10 µl of BT-11 Amplification Control + 40 µl of Bt-11 pat gene amplification reaction mixture. **Negative Controls** should be prepared with 10 µl of sterile bidistilled water and the corresponding amplification reaction mixture.

Proceed to Amplification Area

6.- Close amplification vials and place them in thermal cycler. Store remaining of all reagents at -15±8 °C.

NOTE

Check thermal cycler regularly. Non-existent or poor calibration of the equipment may render equivocal results.

Perform the amplification according to the following programs:

	35S	Maize	Bt-11 <i>pat</i> gene
INITIAL DENATURING	94°C / 3 min	94°C / 10 min	94°C / 10 min
CYCLIC AMPLIFICATION	94°C / 30 sec	94°C / 30 sec	94°C / 30 sec
	55°C / 30 sec	70°C / 30 sec	65°C / 30 sec
	72°C / 45 sec	72°C / 30 sec	72°C / 45 sec
NUMBER OF CYCLES	45	40	50
FINAL ELONGATION	72°C / 3 min	72°C / 10 min	72°C / 10 min

NOTE

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).

6. INTERPRETATION OF RESULTS

The analysis of amplification products is performed by horizontal electrophoresis in low EEO-agarose gels (e.g. MB Agarose, Cat. No. 20.011/2). Band visualisation is improved in 2 % gels using TBE 0.5X as running buffer or 3 % gels using TAE 1X as running buffer. It is recommended to add ethidium bromide in the agarose gel for a better resolution and visualisation. Load 10-20 µl of the amplification product, and proceed to electrophoresis. Due to the small size of the obtained bands⁵, special care must be taken so that separation between products and primer dimers is achieved, while avoiding band migration to the end of the gel.

NOTE

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

Result for positive samples is as follows:

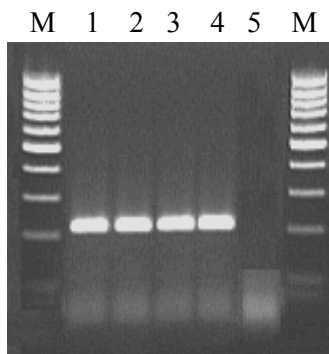
35S Amplification Reaction Mixture	226 bp
Maize Amplification Reaction Mixture	225 bp
Bt-11 <i>pat</i> gene Amplification Reaction Mixture	189 bp

Detection of 35S promoter is enough to classify a sample as GMO positive. However, classification as Bt-11 maize positive must render a positive result for the *pat* gene.

⁵ Small sizes for amplification products are essential for a good detection in food samples, where DNA is highly fragmented, with average size values of around 400-500 bp. Therefore, a detection method must take this fact into account, in order to avoid false negatives that may arise due to using amplification methods rendering products of higher size than average DNA fragments in food samples.

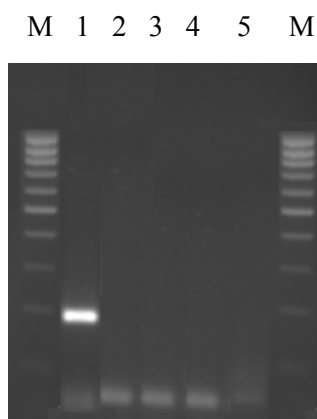
Figure 1: Analysis of the Bt-11 Yield-Gard Maize in different GMO samples. M: 100 bp molecular ladder (Cat. No 31.006). Lane 1: Bt-11 Yield-Gard maize positive (*pat* gene positive). Lanes 2-4: Bt-11 Yield-Gard maize negative (*pat* gene negative) but GMO positive samples. Lane 5: amplification blank.

A) Results obtained using the **35S Amplification Reaction Mixture**



35S Promoter (226 bp)

B) Results obtained using the **Bt-11 *pat* gene Amplification Reaction Mixture**



***pat* gene (189 bp)**

7. 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 Controls must render the corresponding bands (see 'Interpretation of Results' chapter). 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.

8. PROCEDURAL PRECAUTIONS

1. Laboratory workflow must be unidirectional, from pre-amplification area to 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.
18. Biotoools laboratories participate in a regular and satisfactory way in intercomparison studies (ring tests) recognised internationally (USDA-GIPSA, Gemma Scheme, FAPAS & FEPAS etc.). On the other hand, Biotoools is an active member of different standardization and regulation committees (AENOR, CEN).

9. 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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