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BIOGENICS Kits

Kits for detection of GMOs in food and food materials

RoundUp Ready® SOYA IDENTIFICATION KIT

Ref. 91.222

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

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) by 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).

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[1] (we recommend using Speedtools Food DNA Extraction Kit and/or Speedtools Plant DNA Extraction Kit, 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 / µl, $A_{260/280}$=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.

BIOGENICS SOYA kit is based on the detection and amplification of gene regions specific for RoundUp Ready® soya, and not present in native soya (EPSP synthase gene from *Agrobacterium tumefaciens*), as well as GMO generic sequences (35S promoter[2]), present in approximately 90 % of the GMOs that have been commercialised up to date. Also, control amplifications are included, so that discrimination between real negatives and false negatives due to amplification inhibition is achieved.

BIOGENICS SOYA kit can be used with heterogeneous samples (more than one component), and detects presence of RoundUp Ready® soya, as well as GMOs containing 35S promoter. However, it does not detect GMOs containing only NOS terminator.

REAGENTS

The Kit contains amplification reagents in liquid format for performance of 48 amplification reactions (Ref. 91.222). Thaw and handle reagents on ice.

- **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 $MgCl_2$ and DNA polymerase, in the adequate ratios.

  - **35S Master Mix**: for the 35S promoter identification
    which indicates GMO presence.
  - **EPSP Master Mix**: for the RoundUp Ready® soya identification
    *which indicates RoundUp Ready® soya presence –**EPSP synthase gene**–*
  - **Soya Master Mix**: for the soya identification
    *which indicates soya presence – **lectin gene**, present both in native and GMO soya –*

  Store at –15±8 ºC. Thaw and handle on ice. Do not freeze/thaw repeatedly. For frequent use, prepare aliquots of the vial contents

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

[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 eliminates 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.

- **DNA Polymerase** (1U/µl) Two vials: 2 x 130 µl
  Store at –15±8 ºC. Add to reaction mixtures shortly before introduction of vials in thermal cycler.

- Control DNAs
  - **ROUND-READY SOYA Amplification Control**:
    ROUND-READY Soya control (positive to EPSP synthase gene and 35S promoter). DNA amplification products containing DNA sequences from EPSP synthase gene (10^6 copies /µl) and 35S promoter (10^6 copies /µl).
  - **SOYA Amplification Control**:
    Soya control (positive to lectin gene). DNA amplification product containing DNA sequences from lectin gene (10^6 copies /µl).

  Store at –15±8 ºC. Thaw and handle on ice. Do not freeze/thaw repeatedly. For frequent use, prepare aliquots of the vial contents.

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 pipettes[3] (10, 20 and 200 µl), filter or positive displacement tips, RNase-free[4]
- 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[5] 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 (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

[3] Precision of automatic pipettes must be in the range of 3 % of the indicated volume. If necessary, callibrate 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.
[5] Available in our catalogue (Ref. 40.201).

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 / googles 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-existant or poor calibration of the equipment may render equivocal results.

1.- Final reaction volume is 50 µl. Calculate the necessary volume of the **corresponding 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 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, $MgCl_2$** 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 + $MgCl_2$ + DNA Polymerase + sterile bidistilled water) on ice:

Reagent	35S	Soya	EPSP
$MgCl_2$ Solution	2.5 µl	2 µl	
Master Mix	15 µl		
Polymerase	1.4 µl	1 µl	1.4 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 25-50 ng from DNA from the purified samples and/or controls to each amplification vial. Complete up to 50 µl final volume.

5.- Close amplification vials. Place them in thermal cycler. Store reagents at –15±8 ºC.

Perform the amplification according to the following program:

	35S	Soya	EPSP synthase
Initial denaturing	94ºC / 3 min	94ºC / 10 min	94ºC / 10 min
Cyclic amplification	94ºC / 30 sec	94ºC / 30 sec	94ºC / 30 sec
	55ºC / 30 sec	60ºC / 30sec	59ºC / 30 sec
	72ºC / 45 sec	72ºC / 1 min	72ºC / 45 sec
Number of cycles	45	40	50
Final elongation	72ºC / 3 min	72ºC / 3 min	72ºC / 3 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).

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 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 bands[6], 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:

35 S Master Mix	226 bp
Soya Master Mix	118 bp
EPSP Master Mix	172 bp

Detection of 35S promoter is enough to classify a sample as GMO positive. However, classification as RoundUp Ready® soya positive must render a positive result for the EPSP synthase gene.

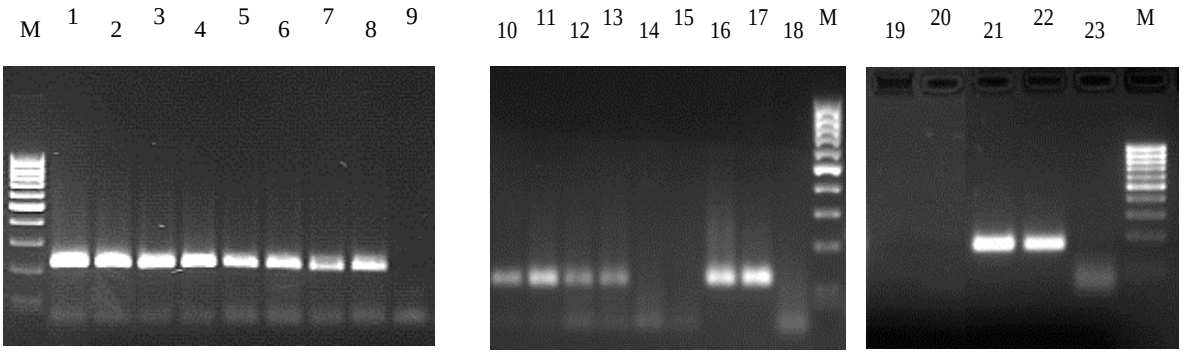

Figure 1: Duplicate analysis of the 35S Promoter, the Soya Control and the EPSP Synthase amplification products of different GMO samples, using in each case 10 and 15 µl of a 10^{-1} dilution of the DNA extraction as amplification templates. M: 100 bp molecular ladder. Lanes 1, 2: anonymous 35S-positive soya beans. 3, 4: anonymous 35S-positive soya flour. 5, 6: anonymous 35S-positive maize grains. 7, 8: 35S-positive RoundUp Ready® Soya Standard Control supplied with the kit. 9: 35S negative control (no DNA). 10, 11: anonymous soya beans. 12, 13: anonymous soya flour. 14, 15: anonymous Soya Control-negative maize grains. 16, 17: Soya Control-positive Soya Internal Control supplied with the kit. 9: Soya-Control negative control (no DNA). 19, 20: EPSP Synthase-negative 35S-positive transgenic soya (the same sample as in lanes 1, 2). 21, 22: EPSP Synthase-positive RoundUp Ready® Soya Standard Control supplied with the kit. 23: EPSP-Synthase negative control (no DNA).

[6] 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.

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.

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. 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

11. 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

12. False negative results may be obtained due to polymerase inhibition. It is recommended to perform control reactions to distinguish between inhibition and true negatives.

13. Cross contamination between samples and exogenous DNA can only be avoided by following good laboratory practice. Instructions in this document must be strictly followed.

14. Use of this product is limited to qualified professional personnel, experienced in DNA purification and DNA amplification techniques.

15. It is important to pipet the indicated amounts, and mix well after each reagent addition. Check pipettes regularly.

16. Biotools 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, Biotools is an active member of different standardization and regulation committees (AENOR, CEN).

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, misuse, results of the use or inability to use any product.

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