



BIOTOOLS

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

BIOGENICS QL Kits

*Kits for detection of GMOs in food and food materials
by Real-Time DNA amplification*

STANDARD KIT

**Equipment: Roche Light Cycler
Cat. No. 91.402L**

Instructions for Use

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

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 by Real-Time amplification.

BIOGENICS QL – Standard Kit detects the presence or absence of genetic sequences present in approximately 90 % of GMOs commercialised up to date. For this purpose, two regions are amplified: *rbcL* gene from chloroplast, serving as control to confirm presence of amplifiable DNA, and 35S promoter from cauliflower mosaic virus (CAMV)¹, detected in all GMOs.

Real-Time monitoring of amplification products is performed by fluorescence reading after incorporation of SYBR® Green in double-stranded DNA molecules obtained after each amplification cycle. Positivity of the sample to 35S promoter is checked by a melting curve (T_m) after amplification.

The sensitivity of the Kit 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 EU threshold (1829/2003 and 1830/2003).

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.

2. 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 Cat. No. 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/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 QL STANDARD Kit can be used with heterogeneous samples (more than one component), and detects presence of GMOs, but does not identify the specific GMO present in the sample.

3. REAGENTS

Kit contains enough reagents in liquid format to perform 48 rxns (Cat. No. 91.402L). 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. 91.401L) contains one set of 24 reactions. Store all reagents at -15±8 °C (except diluted SYG solution which should be stored at 4 °C). Thaw and handle on ice. Do not freeze/thaw repeatedly. For frequent use, we recommend the aliquoting of the vial contents. Mix well the reagents before use.

- **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** Two vials: 2 x 405 μl
35S promoter identification (indicating GMO presence)
 - **PLANT MASTER MIX** Two vials: 2 x 405 μl
plant identification (control reaction, indicating plant presence – chloroplast *rbcL* gene, present both in native and GMO plants)
- **MgCl₂ SOLUTION (50 mM):** Two vials: 2 x 1.8 ml
Mix well before use.
- **DNA POLYMERASE:** Two vials: 2 x 25 μl
Add to reaction mixtures shortly before introduction of vials in thermal cyclers.

¹ 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.

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.

- **INTERNAL CONTROL:** Two vials: 2 x 80 µl
DNA amplified products containing a sequence of *rbcl* gene and a sequence of 35S promoter at a concentration of 10⁶ copies/µl each.
- **SYG:** Two vials: 2 x 0.35 µl
A concentrated solution containing the intercalating dye SYBR Green I. **TO BE DILUTED WITH 500 µl OF SYG BUFFER. Avoid exposure to light.** Add diluted SYG solution to reaction vials shortly before introduction in thermal cycler. **Once diluted SYG vial should be stored at 4 °C.**
- **SYG BUFFER:** Two vials: 2 x 600 µl
For diluting concentrated SYG vial.

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
- 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-Tor⁵ or equivalent, in order to remove DNA from working surfaces

Amplification area

- Real-Time thermal cycler: Roche Light Cycler. Use of this kit in other equipment has not been tested. For further information, contact our Technical Dpt. (info@biotools.eu).
- Laminar flow cabinet
- Racks for reaction vials
- Real-Time amplification vials (as per manufacturer's instructions).
- 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, 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.

⁵ Available in Biotools' catalogue (Cat. No. 40.201).

5. PROTOCOL

A. PREPARATION OF REACTION MIXTURES AND SAMPLES

In order to obtain the best results, **it is essential to keep the reaction vials refrigerated until their introduction in the thermal cycler**. For standard conical vials introduce them on ice or on refrigerating blocks, being careful not to wet the optical cap. Use of reaction mixtures and vials in non-refrigerated conditions may cause a drastic decrease in sensitivity and quality of the obtained fluorescence curves.

1.- Dilute SYG vial as indicated in "Reagents Section".

NOTE

Before starting the procedure check that all reagents contain enough volume to perform the necessary reactions. Thaw all vials on ice and Keep them on ice while on use.

SYG vial must be protected from light. Its exposure to light may inactivate it and affect the performance of the kit dramatically.

2.- Final reaction volume is 25 μ l (Amplification Reaction Mixture + purified DNA). Prepare each **Reaction Mixture (35S and PLANT)** following the table bellow (Table 1) in separates 1.5 ml vials, 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 each Amplification Reaction Mixture for n+1 reactions. **Protect SYG vial from light at all times.**

Table 1. Preparation of Reaction Mixtures (35S and PLANT)

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

Reagent	PLANT REACTION MIXTURE (for <i>rbcL</i> gene)	35S REACTION MIXTURE (for 35 S Promoter)
Master Mix	15 μ l	15 μ l
SYG (diluted)	0.2 μ l	0.2 μ l
DNA Polymerase	0.5 μ l	0.5 μ l
MgCl ₂	1 μ l	1 μ l
Sterile bidistilled H ₂ O	5.8 μ l	5.8 μ l

3.- Aliquot 22.5 μ l of reaction mixture in each amplification vial⁶, **in the laminar flow cabinet.**

4.- Remove tubes from the laminar flow cabinet. **Addition of DNA to reaction mixtures should be performed in the pre-amplification area in order to avoid contaminations (never introduce DNA in the laminar flow cabinet).** Add 2.5 μ l of DNA purified from samples (sample DNA concentration 20 ng/ μ l) and/or controls to each amplification vial. For samples where DNA cannot be quantified it is recommended to standardise a fixed volume to add (1-8 μ l) keeping the proportions of each component of the reaction mixture by reducing the content of sterile bidistilled H₂O.

5.- **Plant Positive Control** should be prepared by adding 2.5 μ l of Internal Control vial + 22.5 μ l of Plant Reaction Mixture. **35S Positive Control** should be prepared by adding 2.5 μ l of Internal Control vial + 22.5 μ l of 35S reaction mixture. **Negative Controls** should be prepared with 2.5 μ l of sterile bidistilled water and the corresponding reaction mixture.

NOTE

Amplification must be started in the next 10 minutes after adding the purified DNA and controls to the amplification mix.


6.- Close amplification vials. When using optical caps, be careful not to leave traces of powder, dust, etc. Place vials in thermal cycler. Store remaining of all reagents at the indicated temperatures.

⁶ Amplification vial will be that specifically recommended by each Real-Time thermal cycler manufacturer (optical cap 0.2 ml tubes, capillaries, etc.).

B. PROGRAMMING THE ROCHE LIGHTCYCLER

rbcl and *35S promoter* amplifications are performed with the same program.

1.- Ensure that the LightCycler apparatus is switched on and is properly connected to the computer. For information on the installation of software and instrumentation setup, please consult the operator's manual.

2.- Click on the **Light Cycler Front Screen** icon.  This will bring up the Front Screen menu bar. Choose the **Run** option and the **programming screen** will appear. All of the thermal cycler programming steps are entered from this screen.

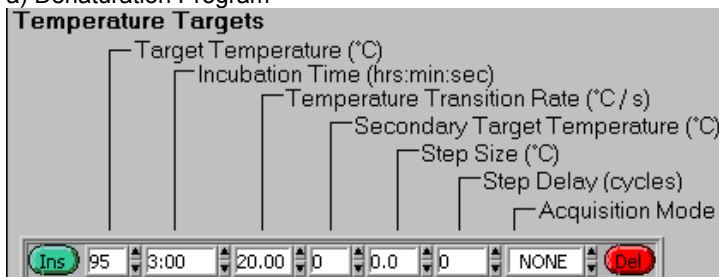
3.- First, define the cycle programs in the cycle program field at the top right of the programming screen. To add a new cycle, click the **Add** button. A dialogue box will appear where the cycle is named. Add the four cycles, **Denaturation**, **Amplification**, **Melting**, and **Cooling**, as shown below

Cycle Program Field

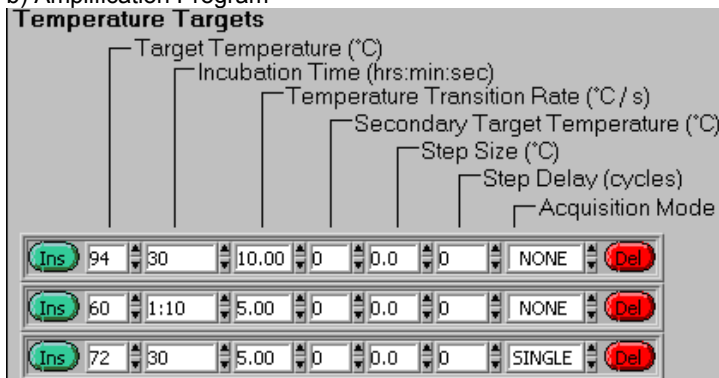


4.- Establish the Cycle Program for each cycle in the "Cycle Program Data Field". The values are entered as shown below, and are also summarised in the following table. Ensure that the instrument only acquires the appropriate fluorescence channel (Sybr) only during the amplification step at 72°C and during the Melting cycle.

a) Denaturation Program



b) Amplification Program



c) Melting Program

Temperature Targets

Target Temperature (°C)
 Incubation Time (hrs:min:sec)
 Temperature Transition Rate (°C / s)
 Secondary Target Temperature (°C)
 Step Size (°C)
 Step Delay (cycles)
 Acquisition Mode

Ins	72	1:30	20.00	0	0.0	0	NONE	Del
Ins	95	1	20.00	0	0.0	0	NONE	Del
Ins	60	15	20.00	0	0.0	0	SINGLE	Del
Ins	98	1	0.10	0	0.0	0	CONT	Del

d) Cooling Program


Temperature Targets


Target Temperature (°C)
 Incubation Time (hrs:min:sec)
 Temperature Transition Rate (°C / s)
 Secondary Target Temperature (°C)
 Step Size (°C)
 Step Delay (cycles)
 Acquisition Mode

Ins	40	30	20.00	0	0.0	0	NONE	Del
-----	----	----	-------	---	-----	---	------	-----

Thermal Cycler Parameters

Denaturation: 1 cycle			
Temperature	Time (sec.)	Slope (°C/sec)	Acquisition Mode
95 ° C	180	20	None
Amplification: 35 cycles			
Temperature	Time (sec.)	Slope (°C/sec)	Acquisition Mode
94° C	30	10	None
60° C	70	5	None
72° C	30	5	Single
Melting: 1 cycle			
Temperature	Time (sec.)	Slope (°C/sec)	Acquisition Mode
72 ° C	180	20	None
95 ° C	1	20	None
75 ° C	15	20	Single
98 ° C	1	0.1	Continuous
Cooling : 1 cycle			
Temperature	Time (sec.)	Slope (°C/sec)	Acquisition Mode
40	30	20	None

5.- Click the "Edit Samples" button . The Loading Screen will appear. In this window the Sample Names are entered. The number of sample to be analysed is designated in the "Maximum Position" field. When completed, click the done "Done" button.

6.- Select the Run button  to begin the thermal cycle instrument. A dialogue box will appear were you name the file and chose where the data will be stored.

6. INTERPRETATION OF RESULTS

Use of thermal cyclers with coupled fluorescence capture systems allows the monitoring in real time and in each amplification cycle of the generated amplification products. Interpretation of results will be based on the specific software for the used equipment, following the manufacturer's instructions and recommendations.

Fluorescence curves

Real-Time amplification systems will render fluorescence increase curves depending on the amplification cycle. Thus, positive samples will render an increasing fluorescence curve. Cycle where fluorescence increase starts is proportional to the number of target copies present in the sample. On the contrary, negative samples will not render a fluorescence increase during the process, rendering a basal fluorescence line.

The interpretation of results is performed based on the melting curve analysis.

- There should not be melting curves for Negative Controls (Amplification and DNA purification blanks).
- Samples with Plant Reaction Mixture - if samples contains plant DNA a melting peaks at 85 °C should appear due to *rbcl* gene amplification.
- Samples with 35S Reaction Mixture- if no amplification is observed the sample is negative to GMO; if a melting peak is observed at 87.5 °C the sample is positive to GMO.

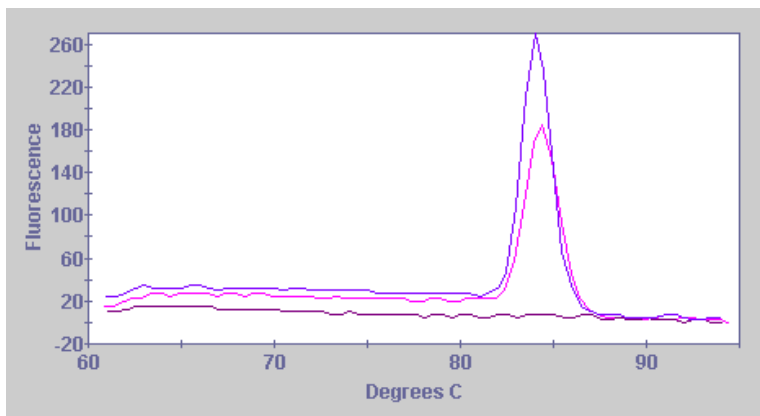


Figure 1. Tm analysis for *rbcl* gene using Plant Reaction Mixture.

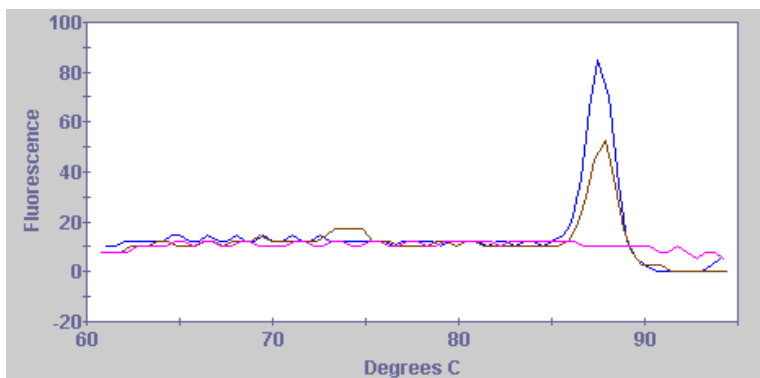


Figure 2. Tm analysis for 35S promoter using 35S Reaction Mixture.

NOTE

It must be considered that the test detects DNA and the concentration of DNA is not always correlated with the percentage of target material, specially in processed samples, or materials containing trace amounts of DNA.

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 peaks in the Melting Analysis (see 'Interpretation of Results' chapter). Vials containing negative control (sterile bidistilled water) must render no peaks. 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 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.
18. 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 standardisation 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, 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.
Valle de Tobalina – 52 – Nave 39, 28021 Madrid – Spain

BIOTOOLS® is a registered mark of BIOTOOLS, Biotechnological & Medical Laboratories, S.A.
Termi-DNA-Tor™ is a trademark of BIOTOOLS, Biotechnological & Medical Laboratories, S.A.
Genomic DNA extraction™ Kit is a trademark of BIOTOOLS, Biotechnological & Medical Laboratories, S.A.

SYBR® Green is a registered trademark of Molecular Probes Inc.
LightCycler® is a registered trademark of a member of the Roche group.



© 2007 BIOTOOLS, Biotechnological & Medical Laboratories, S.A. All rights reserved