If you have any further questions about this test-kit, please contact:

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Additional available PCR*-kits:

EHEC-ToxinGene Detection Kit **Product Code: EHEC-E**
for genotypical detection of eae-gene of enterohemorrhagic *E. coli*

EHEC-ToxinGene Detection Kit **Product Code: EHEC-S**
for genotypical detection of slt-I and slt-II genes of enterohemorr-hagic *E. coli*

HAVGene Detection Kit **Product Code: HAV**
for genotypical detection of Hepatitis A-virus

MaizeGene Detection Kit **Product Code: MAIZE**
for genotypical detection of Bt-Maize

WheatGene Detection Kit **Product Code: WHEAT**
for genotypical detection of common wheat in durum wheat products

35-SGene Detection Kit **Product Code: 35-S**
for genotypical detection of 35-S Promotor gene

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SOY e 22.03.01

SoybeanGene Detection Kit

for Genotypical Detection of
Roundup Ready™ Soybean

Product Code: SOY

For in-vitro use only!

mediagnost
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コスモ・バイオ株式会社

Intended Use

Determination of genetically modified Roundup Ready™ soybeans using polymerase-chain reaction (PCR*). The reaction mixes are optimized for use with Taq-Polymerase from Roche Diagnostics GmbH.

Assay Principle

This SoybeanGene Detection Kit is suited for the determination of genetically modified Roundup Ready™ soybean, which is authorized as food and fodder in the European Union. This soybean carries a hybrid EPSPS-gene as a transgene, which is responsible for the resistance against the herbicide Glyphosphate. The rapid test method polymerase-chain reaction (PCR*) allows the specific and highly sensitive determination of definite DNA-sequences, defined through selection by primers. PCR* forms the basis of detecting genetically engineered material in a sample. The SoybeanGene Detection Kit fulfills the requirements on research methods, that are listed in "Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG" (23.01.22, März 1998) for the determination of change in soybeans by means of genetic engineering. The SoybeanGene Detection Kit uses two different pairs of primers to determine the **transgene** in Roundup Ready™ soybean and the **lectin-gene** respectively. Reaction Mix RR-Soy amplifies a **172 bp-fragment of the transgene** and Reaction Mix Lectin amplifies a **118 bp-fragment of the lectin-gene.** The lectin-gene is detectable in natural soy as well as in genetically modified soy. A positive test-result after the use of Reaction Mix Lectin with a DNA-sample is necessary to control the DNA-extraction of the sample material. This control proves a successful extraction of amplifiable sample-DNA out of the examination material. The Control-DNAs in the kit serve as a check for successful PCR-reaction. Carrying along negative-controls to check for and to exclude possible contamination during the test is highly recommended.

Target-DNA and Amplified Fragments

Transgene in Roundup Ready™ soy
Lectin-gene

RR-Soy	reaction-amplicon:	172 bp
Lectin	reaction-amplicon:	118 bp

Kit Contents

Reaction Mix (Rx-Mix) RR-Soy	for 5 x 10 assays	5 x 225 µl
Reaction Mix (Rx-Mix) Lectin	for 5 x 10 assays	5 x 225 µl
Control-DNA RR-Soy		1 x 50 µl
Control-DNA Lectin		1 x 50 µl
DNA-Standard		1 x 200 µl
Sample Buffer		1 x 250 µl
Sterile H_2O		1 x 500 µl

Except the Taq-Polymerase, the kit contains all necessary reagents for running 50 specific PCR*-amplifications to determine the genetically modified Roundup Ready™ soybean and for running 50 specific PCR*-amplifications for lectin-gene to verify the DNA-extraction out of the respective material. Furthermore, it contains the respective Control-DNA to verify the reaction, a DNA-Standard for size-comparison of the amplified products and Sample Buffer to apply the amplified DNA-products on an agarose-gel.

Required Materials Not Provided

- Taq-Polymerase (1 unit/µl) from Roche Diagnostics GmbH (0.5 U/assay required)
- Agarose for manufacturing a 2% agarose-gel
- TAE- or TBE-electrophoresis buffer
- Ethidiumbromide for gel-staining
- Mineral oil (for thermocyclers without heating-lid)

Storage

The kit and its contents should be stored at -20°C.

Sample Preparation

Suitable methods for nucleic acid extraction should be used for DNA-preparation.

Precautions

PCR* is the most sensitive method to determine DNA-molecules. Therefore it is necessary to take special precautions to avoid false-positive results:
⇒ separation of sample-preparation, undertaking of PCR* and detection
⇒ working with sterile disposable gloves
⇒ use of sterile disposable material
⇒ use of filter-dispenser tips

PCR*

The PCR*-reaction is carried out as a 25 µl assay in sterile thin 0.2 ml reaction vessels. Thaw the required amount Reaction Mix on ice, vortex and centrifuge the aliquot before use.

CAUTION: Avoid repeated thawing and freezing cycles of Rx-Mix-aliquots (**max. 2 cycles**).

It is highly recommended to carry along a **Negative Control** (water instead of sample-DNA) and a Positive Control (Control-DNA instead of sample-DNA) in each PCR*-assay (one Negative Control (water) per 10 samples is suggested).

Assay Procedure

1. Thaw the required amount of Rx-Mix RR-Soy and Rx-Mix Lectin, mix it with a vortex-mixer and centrifuge

2. Add Taq-Polymerase[t], mix and centrifuge

Activation of Reaction Mix RR-Soy:

Reaction Mix RR-Soy for 10 samples (1 aliquot Rx-Mix RR-Soy)	220 µl
Taq-Polymerase[t] (1U/µl)	5 µl
⇒ activated Reaction Mix RR-Soy	⇒ 225 µl

[t] Taq-Polymerase Roche Diagnostics GmbH

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Activation of Reaction Mix Lectin:

Reaction Mix Lectin for 10 samples (1 aliquot Rx-Mix Lectin)	220 µl
Taq-Polymerase[t] (1U/µl)	5 µl
⇒ activated Reaction Mix Lectin	⇒ 225 µl

[t] Taq-Polymerase Roche Diagnostics GmbH

3. Pipette 22.5 µl activated Reaction Mix per sample and control reaction in a labeled thin 0.2 ml reaction vessel under PCR*-conditions

4. Add 2.5 µl sample-DNA, water or Control-DNA respectively

CAUTION: Pipette the Positive Control-DNA always last. Already smallest traces of positive DNA may cause contamination and lead to false-positive results!

Flow Chart of Assay Protocol (10 samples):

RR-Soy	Activ. Rx-Mix RR-Soy	H_2O	Sample-DNA	Control-DNA RR-Soy
Negative Control	22,5 µl	2,5 µl	----	----
Sample 1-8	22,5 µl each	----	2,5 µl each	----
Positive Control RR-Soy	22,5 µl	----	----	2,5 µl
Lectin	**Activ. Rx-Mix Lectin**	**H_2O**	**Sample-DNA**	**Control-DNA Lectin**
Negative Control	22,5 µl	2,5 µl	----	----
Sample 1-8	22,5 µl each	----	2,5 µl each	----
Positive Control Lectin	22,5 µl	----	----	2,5 µl

5. Apply a layer of 20 µl sterile mineral oil on each sample. (This is not necessary when using a thermocycler with heating-lid)

6. Place the samples in the thermocycler and start the amplification:

Initial Denaturation		3 min. 94°C
Cycling 40 x	denaturation	0.5 min. 94°C]
	annealing	0.5 min. 60°C } 40 x
	extension	0.5 min. 72°C]
Final Elongation		5 min. 72°C

7. Mix 12 µl of each sample with 2 µl Sample Buffer

8. Separate the PCR*-samples as well as 14 µl of the DNA-Standard on a 2% agarose-gel (buffer system: 1 x TAE, pH 8.0 or 1 x TBE, pH 8.0)

9. Running time: 15 min
Voltage: 3 - 6 V/cm gel-length

10 Stain the gel with ethidiumbromide (0.5 µg/ml in 1 x TAE or 1 x TBE)

11. Documentation of the gel

PCR*: Hoffmann-LaRoche Inc. owns the U.S.-Patent for performing PCR. The performance of PCR may be carried out under license of Hoffmann-LaRoche Inc.. The provided informations with this test-kit do not include an authorization to perform PCR nor a license for the performance. The user of this kit performs the PCR on his own responsibility to obtain the necessary license.

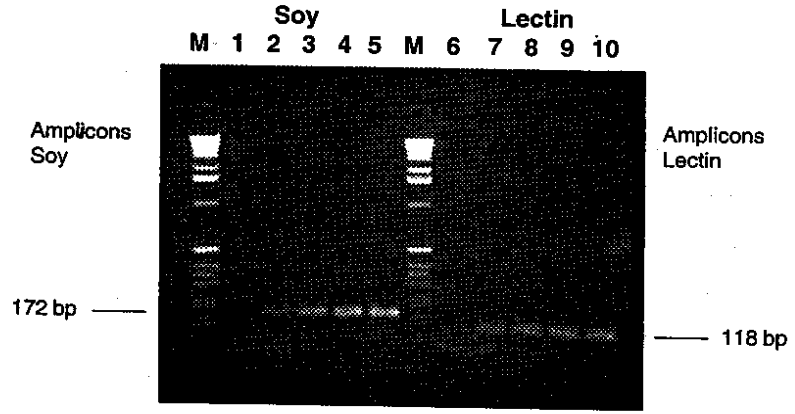

Figure 1: Amplification of Roundup Ready™ soybean and lectin gene sequences respectively with PCR*.

Lane 1: Negative Control (water) Rx-Mix RR-Soy
Lane 2: 2.5 µl sample-DNA, dilution 1:100 Rx-Mix RR-Soy
Lane 3: 2.5 µl sample-DNA, dilution 1:10 Rx-Mix RR-Soy
Lane 4: 2.5 µl sample-DNA, Rx-Mix RR-Soy
Lane 5: Control-DNA RR-Soy

Lane 6: Negative Control (water) Rx-Mix Lectin
Lane 7: 2.5 µl sample-DNA, dilution 1:100 Rx-Mix Lectin
Lane 8: 2.5 µl sample-DNA, dilution 1:10 Rx-Mix Lectin
Lane 9: 2.5 µl sample-DNA, Rx-Mix Lectin
Lane 10: Control-DNA Lectin

Lane M: DNA-Standard

For amplification of Roundup Ready™ Soybean and Lectin-gene sequences using PCR* increasing amounts of sample-DNA were used (Lane 2-4 Rx-Mix RR-Soy, Lane 7-9 Rx-Mix Lectin). Sample-DNA was extracted from soy-flour, containing 1% Roundup Ready™ Soy-flour.