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

SoybeanGene™ Detection Kit **Product Code: SOY**
for genotypical detection of RoundupReady™ Soy

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35-S e 22.03.01

コスモ・バイオ株式会社

35-SGene™ Detection Kit

for Genotypical Detection of the
35-S-Promotor

Product Code: 35-S

For in-vitro use only!

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Intended Use

Determination of transgenes in genetically modified plants under the control of the cauliflower mosaic virus 35S promotor (CaMV 35S) using polymerase-chain reaction (PCR*). The reaction mixes are optimized for use with Taq-Polymerase from Roche Diagnostics GmbH.

Assay Principle

The 35-SGene™ Detection Kit is suited for the determination of genetically modified plants which contain transgenes under the control of the cauliflower mosaic virus 35S (CaMV 35S) promotor as for example Bt-Maize or Roundup Ready™ soybeans. The rapid test method polymerase-chain reaction (PCR*) allows the specific and highly sensitive determination of defined DNA-sequences by the selection of primers. PCR* forms the basis of detecting genetically engineered material in a sample. The 35-SGene™ Detection Kit fulfills the requirements on research methods, that are listed in "Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG" (L.00.00-31, September 1998) for the determination of genetically modified plants.
The 35-SGene™ Detection Kit uses two different pairs of primers to determine parts of the transgene and of the chloroplast genome, respectively. Reaction Mix 35S (**Rx-Mix 35S**) amplifies a **195 bp** fragment of the transgene and Reaction Mix CP (**Rx-Mix CP**) amplifies a **500-600 bp** fragment of the chloroplast genome. This genome is detectable in natural as well as in genetically modified plants. The amplification of the chloroplast genome in a DNA sample is necessary to control the DNA extraction of the sample material. A positive result proves a successful extraction of amplifiable sample DNA out of the examination material. The Control-DNAs in the kit serve as a check for succesful PCR-reaction. Carrying along negative-controls to check for and to exclude possible contamination during the test is highly recommended.

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Target-DNA and Amplified Fragments

Transgenes in genetically modified plants under the control of the CaMV 35S promotor, Chloroplast genome

35S	reaction-amplicon:	195 bp
CP	reaction-amplicon:	500-600 bp

Kit Contents

Reaction Mix (Rx-Mix) 35S	for 5 x 10 assays	5 x 225 µl
Reaction Mix (Rx-Mix) CP	for 5 x 10 assays	5 x 225 µl
Control-DNA 35S		1 x 50 µl
Control-DNA CP		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 transgenes in genetically modified plants under the control of the CaMV 35S promotor and for running 50 specific PCR*-amplifications for parts of the chloroplast genome 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 35S and Rx-Mix CP, mix it with a vortex-mixer and centrifuge

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

Activation of Reaction Mix 35-S:

Reaction Mix 35-S for 10 samples (1 aliquot Rx-Mix 35-S)	220 µl
Taq-Polymerase[t] (1U/µl)	5 µl
⇒ activated Reaction Mix 35-S	⇒ 225 µl

[t] Taq-Polymerase Roche Diagnostics GmbH

Activation of Reaction Mix CP:

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

35-S	Activ. Rx-Mix 35-S	H_2O	Sample-DNA	Control-DNA 35-S
Negative Control	22,5 µl	2,5 µl	----	----
Sample 1-8	22,5 µl each	----	2,5 µl each	----
Positive Control 35-S	22,5 µl	----	----	2,5 µl
CP	**Activ. Rx-Mix CP**	**H_2O**	**Sample-DNA**	**Control-DNA CP**
Negative Control	22,5 µl	2,5 µl	----	----
Sample 1-8	22,5 µl each	----	2,5 µl each	----
Positive Control CP	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		5 min. 94°C
Cycling 40 x	denaturation	0.5 min. 94°C }
	annealing	0.5 min. 57°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.

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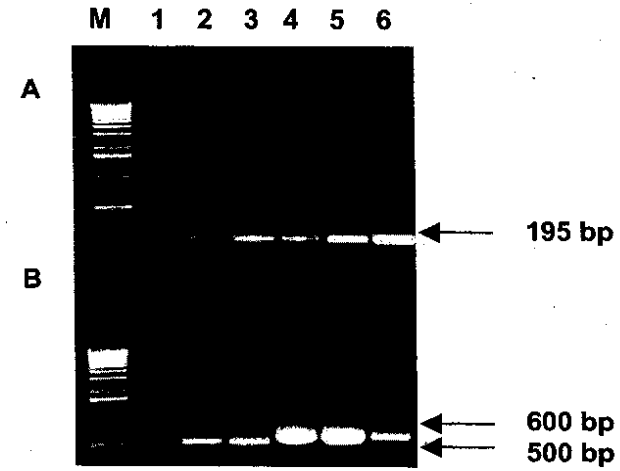

Figure 1: Amplification of RR™ soybean and Bt maize (A) as well as chloroplast (B) gene sequences with PCR*.

(A)
Lane 1: Negative Control (water) Rx-Mix 35S
Lane 2: 2.5 µl sample-DNA, 0,1% Bt-Maize (Rx-Mix 35S)
Lane 3: 2.5 µl sample-DNA, 1% Bt-Maize (Rx-Mix 35S)
Lane 4: 2.5 µl sample-DNA, 0,1% RR-Soya (Rx-Mix 35S)
Lane 5: 2,5 µl sample-DNA, 1% RR-Soya (Rx-Mix 35S)
Lane 6: Control-DNA 35S
(B)
Lane 1: Negative Control (water) Rx-Mix CP
Lane 2: 2.5 µl sample-DNA, 0,1% Bt-Maize (Rx-Mix CP)
Lane 3: 2.5 µl sample-DNA,1% Bt-Maize (Rx-MixCP)
Lane 4: 2.5 µl sample-DNA, 0,1% RR-Soya (Rx-Mix CP)
Lane 5: 2.5 µl sample-DNA, 1% RR-Soya (Rx-Mix CP)
Lane 6: Control-DNA CP

Lane M: DNA-Standard

For the amplification of RR™-Soybean and Bt-Maize gene sequences with the indicated Rx-Mixes sample-DNAs were extracted from reference materials containing 0,1% or 1%genetically modified material.