Microsynth

User Manual

Pentaplex Real-Time PCR AllSoyC

Principle

Detection of genetically modified soybeans by Real-Time polymerase chain reaction (Real-Time PCR) is based on the amplification of a specific region of the transgenic marker. The amplified products are detected simultaneously via fluorescent dyes, each dye is characteristic for one genetically modified soybean or soybean lectin (Le1) gene, respectively. DNA of the following transgenic soybeans and soybean lectin gene can be detected by exciting the corresponding fluorescence dye (ex, max. excitation wavelength [nm]; em, max. emission wavelength [nm]):

Mon87708	**FAM (ex 494 / em 520)**
Mon87769	**JOE (ex 520 / em 548)**
Lectin	**ROX (ex 575 / em 605)**
FG72	**Cy5 (ex 646 / em 662)**
Mon87705	**DY681 (ex 691 / em 708)**

The cycle at which the fluorescence from a dye crosses a given threshold yields the cycle threshold, Ct. Quantification of the amount of specific DNA contained in a sample can be achieved through comparison of the measured Ct to known standards.

Contents and Storage

5 tubes of primer-probe mix, lyophilized, for 5x20 reactions. Shipped at ambient temperature, store at -20°C, do not expose to light.

Reagents to be Supplied by User

PCR Mastermix, e.g QuantiTect Multiplex PCR NoROX from Qiagen (Cat.no. 204743), Sensifast Probe Multiplex PCR Master Mix (Cat. no. BL-BIO-86020) from Bioline or similar product.

Protocol

1. Add 150 µl water (PCR grade) per tube of primer-probe mix, vortex vigorously and incubate for 5 min at 60°C (store solution at 4°C, do not expose to light, stable for 1 week).

2. Add 250 µl 2X PCR Polymerasemix e.g. QuantiTect Multiplex PCR NoROX or respective amount of similar product and mix well.
Yields 400 µl ready-to-use mastermix for 20 reactions à 25 µl reaction volume.

3. Mix 20 µl ready-to-use mastermix with 5 µl sample solution (recommended amount of DNA: 100 ng) in a suitable PCR reaction vessel.

4. Set up your Real-Time PCR machine according to the manufacturer in order to be able to measure the used fluorescence dyes.

5. Use the following thermal cycling profile:

1 15 min, 95°C (QuantiFast: 5 min)
2 10 s, 95°C (QuantiFast: 5 s, 95°C)
3 60 s, 60°C (QuantiFast: 30 s,60°C)
4 Repeat steps 2 to 3 **35 times**

6. Analyze the fluorescence traces according to the manufacturer of your Real-Time PCR machine and determine the Ct-values and the amount of target DNA in each sample by comparing to known standards.

Remark: Please ignore potential upcoming signals after cycle 35.

Contact

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Further Information

https://www.microsynth.ch/food-testing-assays.html

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