Microsynth

User Manual

Duplex Real-Time PCR AllWasabi

Principle

Species detection by Real-Time polymerase chain reaction (Real-Time PCR) is based on the amplification of a specific region of the species' genome. The amplified products are detected simultaneously via fluorescent dyes, each dye is characteristic for one species. DNA characteristic for the following species can be detected by exciting the corresponding fluorescence dye (ex, max. excitation wavelength [nm]; em, max. emission wavelength [nm]):

Horseradish	**FAM (ex 494 / em 520)**
Wasabi	**ROX (ex 575 / em 605)**

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 the ratio of wasabi to horseradish signal (ΔΔCt method), or an external standard series (see below).

Cross-reaction: 100 % wasabi results in a 10 % horseradish signal. In samples with wasabi content < 10 % this should not be a problem. Other cruciferous plants (mustard, rape, radish, broccoli, white cabbage, cauliflower) and turmeric give a horseradish signal of < 1 %.

Contents and Storage

5x HOT FIREPol® Multiplex qPCR Mix from Solis Biodyne (Cat.no. 08-01-00020) or similar product.

Reagents to be Supplied by User

Standards, HOT FIREPol® Probe qPCR Mix Plus from Solis Biodyme (Cat.no. 08-14-00001) 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 HOT FIREPol® Multiplex qPCR Mix 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 5 min, 95°C
2 5 s, 95°C
3 15 s, 60°C
4 Repeat steps 2 to 3 **45 times in total**

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.

Contact

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Further Information

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

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