Microsynth

User Manual

Tetraplex Real-Time PCR AllMilch

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]):

species	fluorophor	target gene
Cow	FAM (ex 494 / em 520)	AY676873
Goat	JOE (ex 520 / em 548)	CytB
Sheep	ROX (ex 575 / em 605)	CytB
Water buffalo	Cy5 (ex 646 / em 662)	CytB

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.Observed cross-reaction: 100 ng goat-DNA leads to a signal of 10 ng sheep-DNA.

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) 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 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
2 10 s, 95°C
3 60 s, 63°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.

Contact

rene.koeppel@klzh.ch
real-time@microsynth.ch

Further Information

www.microsynth.ch/food-testing-kits.html

Kantonales Labor Zürich
Fehrenstrasse 15, Postfach
CH-8032 Zürich
Telefon +41 43 244 71 00
Fax +41 43 244 71 01
www.klzh.ch
Eine Dienstleistung der Gesundheitsdirektion

Microsynth AG
Schützenstrasse 15
CH-9436 Balgach
Telefon +41 71 722 83 33
Fax +41 71 722 87 58
www.microsynth.ch