Microsynth

# User Manual

# Tetraplex Real-Time PCR AllNut

## Principle

This method describes a routine procedure for the quantitative detection of DNA from the allergens listed below using real-time PCR. In addition to the primers specific for each individual system, four differently labeled TaqMan probes enable simultaneous fluorescence-based detection of the four allergens. The use of multicopy target sequences results in a lower limit of detection (LOD):

| | |
|---|---|
| **Hazelnut** | **FAM** |
| **Peanut** | **HEX** |
| **Walnut** | **ROX** |
| **Cashew** | **Cy5** |

The number of cycles at which the measured fluorescence signal exceeds a predefined threshold value determines the Ct value (Cycle Threshold).
Quantification of the samples is achieved using an external calibration series (see below).

## Contents and Storage

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

## Reagents to be Supplied by User

SensiFAST™ Probe No-ROX Kit from Bioline, or other appropriate polymerase mix.
Analyte DNA for preparation of the calibration series.

## Protocol

1. Add of 165 µl PCR-grade water to the supplied primer-probe mix. Allow the pellet to dissolve completely and vortex thouroghly.

2. Add 275 µl of 2X polymerase mix and mix thoroughly.
Yields 440 µl ready-to-use master mix for 20 reactions of 20 µl reaction volume each (including a 10% reserve).

3. Mix 20 µl of the ready-to-use master mix with 5 µl of sample solution in a suitable PCR reaction vessel.

4. Set up your real-time PCR machine according to the manufacturer's instructions to detect the fluorescence dyes used.

5. Use the following thermal cycling profile (specific for SensiFAST™):

1 5 min, 95°C
2 5 s, 95°C
3 15 s, 60°C
4 Repeat steps 2 to 3 for a total of 45 cycles

6. Analyze the fluorescence traces according to the instrument manufacturer's instructions and determine the Ct values and the amount of target DNA in each sample using a mixed dilution series containing all four analytes (calibration series).

## Remark

If you are using a 5X polymerase mix, use 330 µl PCR-grade water and 110 µl 5X polymerase mix instead. If necessary, adapt the thermal cycling profile to the polymerase mix you are using.
Do not set the threshold line too low. Use the linear view to verify the threshold setting.
Verify that color calibration/gain optimization settings are suitable for the instrument.

## Contact

Kantonales Labor Zürich
Dr. René Köppel
rene.koeppel@kl.zh.ch

## Further Information

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

Microsynth AG
Schützenstrasse 15
CH-9436 Balgach
Telefon +41 71 722 83 33
www.microsynth.com

*MV_26/06.1*