Microsynth

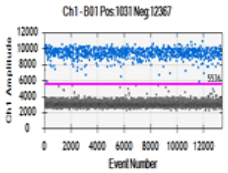
# User Manual

# Duplex Digital Droplet PCR dMarzipan

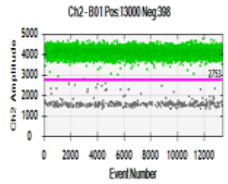
## Principle

The following Method describes the common procedure for the quantitative detection of apricot and prunus species (apricot, almond) DNA by digital PCR.
Two PCR systems specific to apricot and prunus species are used for the amplification. The ratio of the concentrations of positive droplets (copies/ul) together with a conversion factor can be used to determine the weight of the sample in percent by weight.

**Apricot** **FAM**




**Prunus (Apricot, Almond)** **HEX**




## Contents and Storage

4 tubes of primer mix, lyophilized, for 4x24 reactions. Shipped at ambient temperature, store at -20°C
**Supplied on demand:** EXCEL table for results calculation

## Reagents to be Supplied by User

QX200 ddPCR Supermix for Probes 5ml (Cat No. 186-3024) and other consumables for ddPCR with the QX200 system.

## Protocol

1. Add 153 µl PCR grade water per tube of primer-mix, vortex vigorously and incubate for 5 min at 60°C (store solution at 4°C, stable for 1 week).
2. Add 282 µl Supermix for Probes (2x) and mix well.
Yields 435µl ready-to-use Mastermix
3. Mix 17 µl ready-to-use Mastermix with 5 µl sample solution in an appropriate PCR reaction vessel. Recommended amount of DNA: 3 ng, measured by absorbance and/or 1:32 diluted isolated DNA.
4. Use the following thermal cycling profile:

1 10 min, 95°C
2 30 s, 95°C
3 60 s, **55°C**
4 Repeat steps 2 to 3 **50 times in total**
5 10 min, 98°C
7 Hold, 4°C

5. Set the probes parameter for the droplet reading.
6. Analyse the results with QuantaSoft software. To obtain quantifiable results the droplets/µl value for the total amount of DNA (the lowest threshold) should be over 100cp/µl. The value of the empty droplets should be under 100cp/µl.

## Standard Marzipan

To determine the conversion factor, a marzipan product with a known weight and composition can be used.

## Contact

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## Further Information

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

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