Microsynth

User Manual

Digital Duplex PCR dNoBa

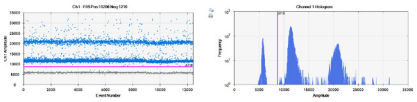
Principle

The following Method describes the common procedure for the quantitative detection of Non-Basmati rice in the samples of the Basmati rice by digital PCR.

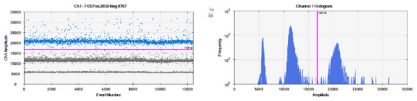
Two PCR systems specific to rice and non-Basmati rice are used for the amplification. The EvaGreen® dye is used for the detection of the PCR product.

Two distinguishable populations of droplets with different final fluorescent signals and one population of droplets without fluorescent signal can be recorded and quantified.

Rice and non-Basmati rice **EvaGreen**



Threshold line for "Total rice" in positive Droplets/µl



Threshold line for "Non-Basmati rice" in positive Droplets/µl

The lowest population refers to negative droplets without DNA (a).

The middle population refers to the rice hk gene and represents the total amount of rice (b).

The top population refers to the non-Basmati rice fraction (c).

If the analysed sample is pure Basmati rice, the top population (c) is not present.

Relative quantification of the non-Basmati rice ratio in percent can be calculated by a simple formula:

$$non-Basmati\ ratio, \% = \frac{100\% \times c}{b}$$

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 EvaGreen supermix 5ml (Cat No.186-4034) and further consumables for ddPCR with 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 EvaGreen Supermix (2x) and mix well.
Yields 435 µl 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: 20 ng, measured by absorbance and/or 1:5 diluted isolated DNA.
4. Use the following thermal cycling profile:

1 5 min, 95°C
2 30 s, 95°C
3 60 s, 60°C
4 Repeat steps 2 to 3 **50 times in total**
5 5 min, 4°C
6 5 min, 90°C
7 Hold, 4°C

6. Set the EvaGreen parameter for the droplet reading.
7. 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.

Contact

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Further Information

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

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