Microsynth

User Manual

Tetraplex Real-Time PCR AllColi

Principle

Simultaneous detection of different enterohemorragic and enteropathogenic Escherichia Coli (E. Coli) strains by Real-Time polymerase chain reaction (Real-Time PCR) is based on the amplification of a specific region of the bacterial DNA. The amplified products are detected simultaneously via fluorescent dyes, each dye is characteristic for a specific toxin or E. Coli. DNA of the following subtypes, which can be detected by exciting the corresponding fluorescence dye (ex, max. excitation wavelength [nm]; em, max. emission wavelength [nm]):

IE. coli spp	**FAM (ex 494 / em 520)**
E. coli eae/intimin	**OE (ex 520 / em 548)**
E. coli stx1	**ROX (ex 575 / em 605)**
E. coli stx2	**Cy5 (ex 646 / em 662)**

Please note :
This kit detects all subtypes , inclusive Stx1d, Stx2f, Stx2g.
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.

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 QuantiFast Multiplex PCR NoROX from Qiagen (Cat.no. 204754) 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 5 min, 95°C
2 5 s, 95°C
3 30s, 60°C
4 repeat steps 2 to 3 **30 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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