primediagnostics

Protocol TaqMan

Technical guidelines

To obtain reliable and reproducible results, the operator should carefully read this entire protocol before running the assay.

- DNA isolation should be performed according the manufacturers DNA isolation protocol.
- Do not use the contents of the kit beyond expire date.
- Do not mix or substitute reagents with those from other lots or sources.
- Avoid reagents warming up to room temperature before and during the use in the assay(s).
- Mix all reagents well and perform a short spin at low g (10 seconds 100g) before use.
- Avoid repeated freezing and thawing.

Supported Reagents

- Primers and probes

Not included media or devices

- PCR plate (VWR Flat 96 well PCR plate 211-0263 or equivalent)
- Filter tips (premium brand: Eppendorf, Merck etc.)
- Optical transparent seal (BIOplastics EU Opti-Seal 157300 or equivalent)
- DNA isolation buffer
- TaqMan grade water (Thermo Scientific HyClone SH30538.01 or equivalent)
- DNA polymerase (TAKARA Premix ex Taq 2x RR003A or equivalent)

Protocol TaqMan

1. Calculate the number of reactions = number of samples + positive control(s) + negative control(s)

2. Dilute kit primers and probe stock 10 times (1 µL stock + 9 µL TaqMan grade water)

3. Prepare MasterMix:
   - Diluted primers and probe (2.5 uL/reaction)
   - Mastermix 12.5 µL
   - Make up to 23 µL TaqMan grade water /reaction

4. Mix well. Perform short spin (10 seconds at 100g)

5. Transfer 23 µL MasterMix/reaction to PCR plate

6. Add 2 µL target /reaction. And add 2 µL Positive Control to PCR plate.

7. Cover PCR plate using an optical transparent seal

8. Mix PCR plate. Perform short spin (10 seconds at 100g)

9. Instrument setup

   HOLD: 95°C for 2 minutes
   CYCLE: 95°C for 15 seconds
   60°C for 1 minute
   } 40 CYCLES

   Optional
   HOLD :4 °C, FOREVER

Contents and storing

A Positive Control (PC) consists of a ready to use specific DNA or RNA sequence (do not dilute the PC). Long time storage of the Positive Control at -20 °C, short term at 4 °C but avoid repeated freeze thaw cycles. When possible (depending on the amount of PC ordered) divide in aliquots and store at -20 °C.

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