AMPLIQON
PCR ENZYMES & REAGENTS

# Fast results using Mic qPCR Cycler and RealQ Plus 2x Master Mix

## RealQ Plus Master Mixes:

- Premixed all-in-one 2x Hot Start solution for qPCR
- High efficiency for accurate experiments
- Reaction set-up at room temperature
- Pre-assembled reactions stable at room temp. > 48 hrs
- High specificity, stability and reproducibility
- Reliable quantification

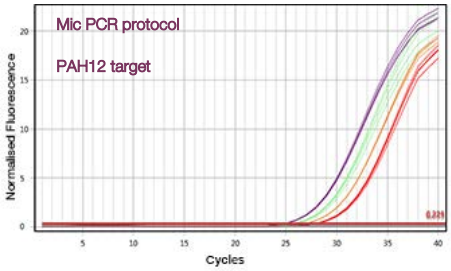
## FAST 2-STEP PROTOCOL

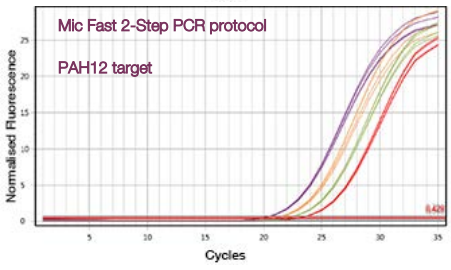
*RealQ Plus 2x Master Mix Green w/o ROX gives excellent results using Mic qPCR Cycler with a fast 2-step PCR protocol*

## RealQ Plus 2x Master Mix Green without ROX provides fast, reliable and quantifiable qPCR results using the Mic qPCR cycler.

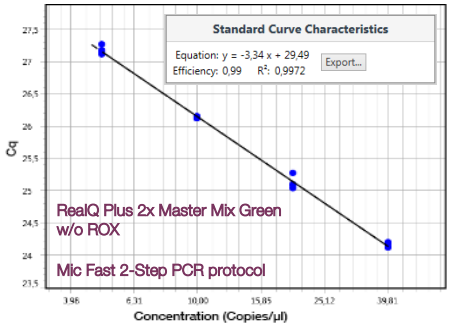
**qPCR Setup:** Apex qPCR 2x GREEN Master Mix w/o ROX, primers for targeting PAH12 (203 bp) and 4 concentrations of gDNA (40 ng, 20 ng, 10 ng and 5 ng). All DNA concentrations were tested in quadruple replicates. The PCR reaction mix was run on Mic qPCR Cycler with settings according to the Mic PCR protocol or Mic Fast 2-step protocol. Melt curve analysis was performed to test specificity. All results were analyzed using the Mic qPCR Cycler software.

### Amplification curves: RealQ Plus Green w/o ROX




### High efficiency using Mic qPCR Cycler




The qPCR standard curve showed a strong linear correlation between the $C_q$ values and log [template DNA]. The efficiency calculated from the curve slope is 99%.

### Mic PCR protocol:

| Cycler step | Temperature | Duration | Cycles |
|---|---|---|---|
| Initial heating | 95 °C | 15 min. | 1 |
| Denaturation | 95 °C | 20 sec. | |
| Annealing | 60 °C | 20 sec. | 35 |
| Elongation | 72 °C | 20 sec. | |
| Melt curve analysis | Instrument default settings | | 1 |

### Mic Fast 2-Step PCR protocol:

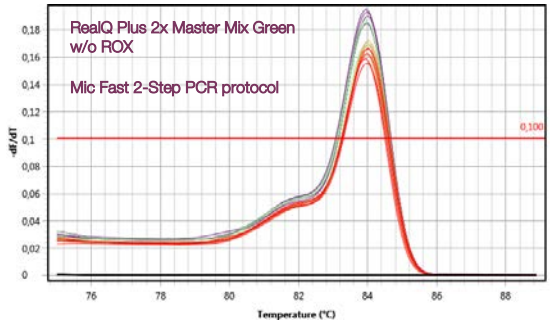
| Cycler step | Temperature | Duration | Cycles |
|---|---|---|---|
| Initial heating | 95 °C | 15 min. | 1 |
| Denaturation | 95 °C | 5 sec. | 40 |
| Elongation | 60 °C | 5 sec. | |
| Melt curve analysis | Instrument default settings | | 1 |

### Cq values for indicated template DNA Concentrations ng/sample

| Mic PCR protocol | 40 ng | 20 ng | 10 ng | 5 ng |
|---|---|---|---|---|
| $C_q$ values | 19.70 | 20.07 | 21.76 | 22.80 |
| | 19.69 | 20.07 | 21.72 | 22.80 |
| | 19.80 | 20.94 | 21.87 | 22.91 |
| | 19.79 | 20.79 | 21.83 | 22.91 |
| Average | 19.745 | 20.7825 | 21.795 | 22.855 |
| SD | 0.058 | 0.113 | 0.068 | 0.064 |

| Mic Fast 2-Step PCR protocol | 40 ng | 20 ng | 10 ng | 5 ng |
|---|---|---|---|---|
| $C_q$ values | 24.18 | 25.03 | 26.15 | 27.11 |
| | 24.11 | 25.09 | 26.12 | 27.17 |
| | 24.19 | 25.07 | 26.12 | 27.27 |
| | 24.14 | 25.27 | 26.13 | 27.14 |
| Average | 24.16 | 25.12 | 26.13 | 27.17 |
| SD | 0.037 | 0.106 | 0.014 | 0,069 |

### High specificity using Mic qPCR Cycler




Melt curve analysis for the amplified pAH12 target. Mic software was used