**Protocol for qPCR Using the Luna Universal qPCR Master Mix (NEB)**

**Reaction Setup:** For best results, **we recommend running each DNA standard and sample in triplicate.**

|  |  |  |
| --- | --- | --- |
| COMPONENT | X1 Reaction | FINAL CONCENTRATION |
| Luna Universal qPCR Master Mix | 10 µl | 1X |
| Forward primer (10 µM) | 0.5 µl | 0.25 µM |
| Reverse primer (10 µM) | 0.5 µl | 0.25 µM |
| Template DNA | 5 µl | < 100 ng |
| Nuclease-free Water | 4µl |  |
| Total Reaction Volume | 20 µl | |

**Real-time PCR condition for GAPDH gene (Reference Gene)**

|  |  |  |  |
| --- | --- | --- | --- |
| Cycles | Temperature | Time | PCR phase |
| 1 | 95oC | 60 Seconds | Initial Denaturation |
| 40 | 95oC | 15 s | Denaturation |
| **40** | 60oC | 30 s | Annealing/ Extension |
| 1 | 60-95°C\* | various | Melt Curve |

**\*Melting curve -Machine (CFX 96 Bio-Rad) default**