|  |  |
| --- | --- |
| Komponente: | Volumen: |
| Supermix | 10 µl |
| frw and rev primers | 1 µl + 1 µl (Final concentration **500 nM**) |
| DNA/Standard | 1 µl |
| Wasser | 7 µl |
| Total: | 20 µl |

Standard: Konzentration 250 ng/ µl ; Volumen 4 µl

Verdünnung:

1:10 – 1:2 – 1:2 -1:2 -1:2

1:10 – 1:20 – 1:40 – 1:80 – 1:160

qPCR-Platte:

S-Standard

P-Probe

NK-Negativ Kontrolle

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | S1 | S3 | NK | P3 | P5 |
|  | S1 | S4 | NK | P3 |  |
|  | S1 | S4 | P1 | P3 |  |
|  | S2 | S4 | P1 | P4 |  |
|  | S2 | S5 | P1 | P4 |  |
|  | S2 | S5 | P2 | P4 |  |
|  | S3 | S5 | P2 | P5 |  |
|  | S3 | NK | P2 | P5 |  |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | -1 | -3 | NK | -3 | -5 |
|  | -1 | -4 | NK | -3 |  |
|  | -1 | -4 | -1 | -3 |  |
|  | -2 | -4 | -1 | -4 |  |
|  | -2 | -5 | -1 | -4 |  |
|  | -2 | -5 | -2 | -4 |  |
|  | -3 | -5 | -2 | -5 |  |
|  | -3 | NK | -2 | -5 |  |

PCR-Programm:

Polymerase Activation & DNA Denaturierung at 95 °C: **3 min**

Denaturierung at 95 °C: **15 sec**

Annealing/Extension + Plate Read at **55 °C: 30 sec**

Cycles: 39 + 1 (wird keine 40 Zyklen laufen, wir haben bei **25 Zyklen gestoppt**)

Melt-Curve Analysis: **5 sec/step**