Quantitative real-time PCR (qPCR)

Sample preparation:

- 5 µL primary virus stock solution
- 7,5 µL DNaseI
- 5 µL $MgCl_2$ [50 mM]
- add 50 µL RNase free water

→ 30 min incubation at 37 °C

→ 10 min enzyme inactivation at 65 °C

Standard curve preparation:

1. Calculation of the plasmid weight

$m_{plasmid}$ = n [bp]* 1 mole/6.096 * 10^{23} * 660 g/mole

$m_{plasmid}$ = n [bp]* 1.096 * 10^{-21} [g/bp]

2. Calculation of the copies of the plasmid

   $m_{copies} = m_{plasmid}$ * copies

   → calculate copies from 1.3 * 10^1 to 1.3 * 10^7 copies

QPCR preparation:

Pipetting scheme for qPCR:

component	**Volume per reaction [µL]**
2 x QuantiFast SYBR Green pcr mix	7.5
Forward primer	1 [10 µM]
Reverse primer	1 [10 µM]
Template	5.0
Rnase free water	0.5
Total volumen	15

- preparing duplicates of standard curve sample and negative control

QPCR protocol:

- two step cycling program

Step	**Time**	**Temperature [°C]**
Inital activation	5 min	95
Denaturation	10 s	95
Annealing/extension	30 s	60

Number of cycles: 45