**First test of MAb:**

Mixes of Taq and MAb made before the setup of the PCR reaction.

| Mab concentration | | | | |
|---|---|---|---|---|
| | 1 µg/ 5U Taq | 0.5 µg/ 5U Taq | 0.1 µg/ 5U Taq | |
| 10xBuffer | 4 | 4 | 4 | µl |
| Taq 40U/µl | 1 | 1 | 1 | µl |
| MAb | 9 | 4,5 | 0,1 | µl |
| H2O | 26 | 30,5 | 34,1 | µl |
| Incubate for 10 min at room temp. | | | | |

Master Mix without Taq

| Fragment: | **Covid19 #7** | | A56C | Date | 19.05.2021 | | | |
|---|---|---|---|---|---|---|---|---|
| PCR volum, µl | 10 | | # of reactions | | 100 | | | |
| | Working | | | | | | | |
| | solutions | | Total volume 1000 µl | | Desired | | | |
| | concentration | | Volum | | concentration | | | |
| H2O | | | 820,00 | | | | | |
| 10X Thermopol #1 | 0 | mM MgCl | 100,00 | | | | | |
| MgCl | 200 | mM | 10,00 | | 2,00001 | mM | | |
| Primer forward | 100 | µM | 3,00 | | 0,3 | µM | | |
| Primer reverse | 100 | µM | 3,00 | | 0,3 | µM | | 94C-2min |
| Fam GC-Clamp | 100 | µM | 0,00 | | 0,00001 | µM | | 94C-20sec |
| dNTP | 100 | mM | 4,00 | | 400 | µM | | A 56C-20sec 45cycles |
| cDNA covid-19 | 100 | ng | 10,00 | | 1 | ng/µl | | 72C-40sec |
| EVA green 20X | | | 40,00 | | 0,8 | X | | Melting |
| BSA | 100 | % | 10,00 | | 1 | % | | |
| Taq | 40 | U/µl | 0,00 | | 0 | U/µl | | |

19µl of the different Taq/MAb mixes where added to 250µl of the master mix above. 0.5 µl Taq (without MAb) was added to the control.

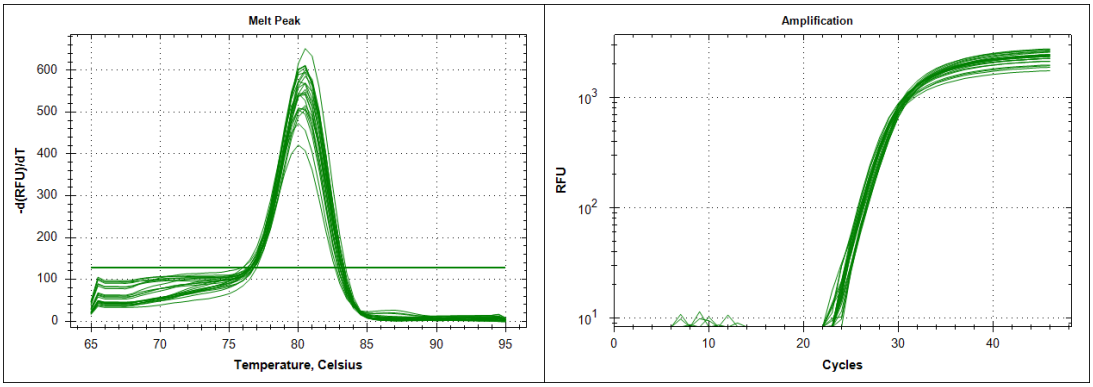
Derivative of the melting curve and the amplification curves are depicted below in the following order. 1µg MAb, 0,5µg MAb, 0,1µg MAb and only Taq (no MAb).

The data show that there are more impurities with reduced MAb concentration. Which also corresponds with the Cq values. The PCR was started with 2 minute incubation at 94°C. This is most likely not enough to denature all the MAb at the highest concentration which is reflected in a high Cq value (needs a few more cycles to get going). This can also increase the variation between the 24 replicates.

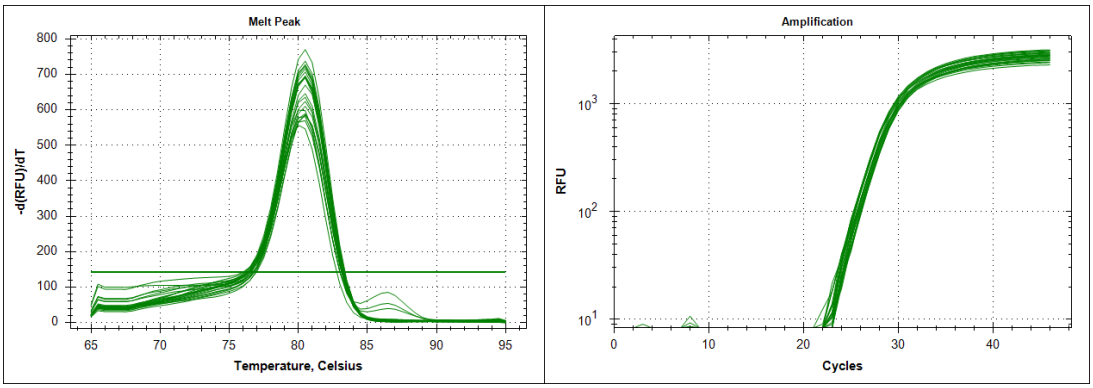
Data summarized in table below.

| | Mean Cq | 1 STD |
|---|---|---|
| 1µg | 26,1 | 0,57 |
| 0,5µg | 25,8 | 0,33 |
| 0,1µg | 25,0 | 0,07 |
| 0µg | 24,4 | 0,12 |

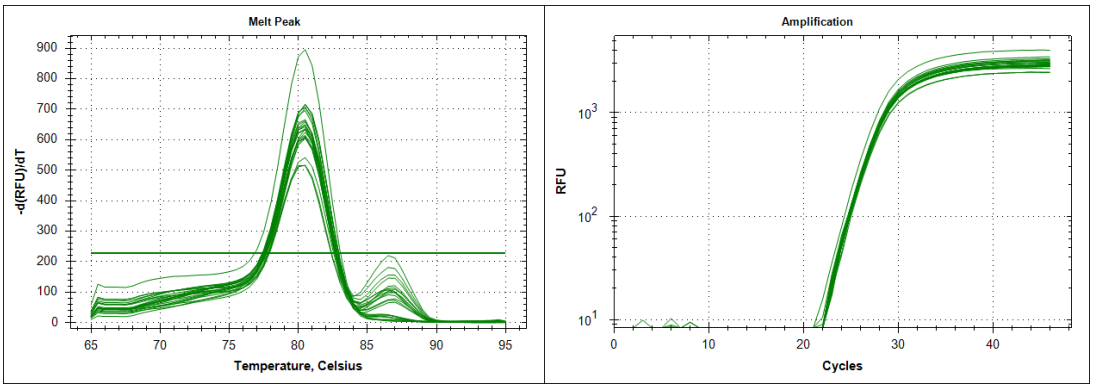
1µg MAb




0,5µg MAb




0,1µg MAb




0µg MAb