# VWR® UNO 96 HPL thermal cycler - the easiest way to avoid evaporation during your PCR!

PCR is a well-established technique to amplify a segment of DNA starting with only a single or a few copies to generate thousands to millions of copies of a particular DNA sequence. A typical amplification reaction includes target DNA, a thermostable DNA polymerase, two oligonucleotide primers, deoxynucleotide triphosphates (dNTPs), reaction buffer and magnesium. Once assembled, the reaction is placed in a thermal cycler to amplify the DNA by series of different temperatures set for amounts of time. To achieve the highest PCR efficiency and robustness, all reaction parameters need to be optimised if the PCR is set-up for the first time. Not only does the chemistry need to be optimised, the thermal cycler is also an important part of the reaction, crucial if a PCR fails or passes. The main parameter to enable robust amplification for a lot of cycles is to control the evaporation during the run. Evaporation can lead to a change in pH, increase in salt concentration, and a decrease in thermal mass. Such a change of chemical composition has the potential to alter the uniformity and robustness of amplification even if the chemistry is set-up correctly.

The data presented in this report will highlight the significance of improved evaporation protection provided by UNO 96 HPL thermal cyclers with regards to obtaining optimal and reproducible PCR conditions.

## MATERIAL AND METHODS

The experiments were performed on the VWR Collection UNO 96 HPL thermal cycler and a thermal cycler made by another manufacturer, both with specific high pressure lids (HPL) to minimise the effect of evaporation. The pressure of both lids is adjustable by the user software and can be realised on every 96 well PCR plate (full skirted, semi-skirted and non skirted). First, 25 µl of PCR master mix was pipetted into all 96 positions of a non skirted PCR plate. Both plates were **not sealed** with foil but closed by a silicone mat (Cat. No. 732-3356) to enable comparable results (no variability during sealing) and increase the probability of evaporation. A very long PCR program with a duration of 4 hours and 35 cycles was used for maximum challenge in the preventing of evaporation. The applied lid pressure was 120 N, because this pressure is the maximum the competitor can produce. In a second PCR experiment we repeated the experiment with the maximum pressure (250 N) the UNO 96 HPL can provide. After the PCR run, the silicone mat was removed and the remaining volume of each well were determined by a pipette.

VWR® COLLECTION

**UNO 96 HPL**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 20 | 22 | 23 | 18 | 23 | 23 | 21 | 21 | 8 | 20 | 25 | 11 |
| B | 22 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 23 |
| C | 22 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 23 |
| D | 21 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 23 |
| E | 13 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 23 |
| F | 22 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 15 |
| G | 22 | 21 | 25 | 24 | 24 | 24 | 23 | 24 | 24 | 24 | 24 | 22 |
| H | 15 | 20 | 20 | 22 | 22 | 21 | 23 | 23 | 23 | 22 | 24 | 20 |

90,79%

**Competitor**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0 | 0 | 0 | 0 | 8 | 10 | 18 | 10 | 0 | 5 | 5 | 0 |
| B | 0 | 2 | 24 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 6 | 0 |
| C | 9 | 16 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 0 |
| D | 4 | 23 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 16 | 10 |
| E | 3 | 23 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 23 | 4 |
| F | 21 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 23 | 3 |
| G | 21 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 10 | 11 |
| H | 21 | 24 | 24 | 25 | 25 | 24 | 25 | 25 | 21 | 3 | 4 | 1 |

74,17%

Key: Remaining volume after a 4 hour run

| 0 – 10 µl | 10 - 19 µl | 20 – 22 µl | 23 – 25 µl |
|---|---|---|---|

**Figure 1.** Remaining volume per well in µl and the average percent sample volume of the entire plate at 120 N lid pressure.

**UNO 96 HPL**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| B | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| C | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| D | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| E | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| F | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| G | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| H | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |

99,78%

Key: Remaining volume after a 4 hour run

| 0 – 10 µl | 10 - 19 µl | 20 – 22 µl | 23 – 25 µl |
|---|---|---|---|

**Figure 2.** Remaining volume per well in µl and the average percent of sample volume in a UNO 96 HPL with 250 N lid pressure.

## RESULTS

Figure 1 shows the individual values of remaining volume due to evaporation over the course of a 4 hour standard PCR thermal profile with a maximum lid pressure of 120 N. The average percent remaining sample volume is specified in the chart above.

Figure 2 shows the individual values of the remaining volume due to evaporation over the course of a 4 hour standard PCR thermal profile with a maximum lid pressure of 250 N (not possible with the competitor). The average percent remaining sample volume is specified in the chart to the left.

## CONCLUSION

With HPL technology the UNO 96 HPL uses a homogenous force that will be created across the entire 96-well plate. Scientists are able to do PCR reactions in any position without losing PCR efficiency, even at the edge of a 96-well plate. With the VWR Collection UNO 96 HPL thermal cycler, reaction volume can be reduced to a minimum. Due to minimal evaporation, no changes in chemical composition of the PCR reaction buffer will occur.