

Low volume real-time PCR with Eppendorf Masterclear™ Cap Strips

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Abstract

Due to the inverted dome of the Eppendorf Masterclear Cap Strips the vapor volume inside the 0.2 mL PCR tubes is noticeably reduced, thus allowing for PCR reaction volumes as low as 2.5 µL.

Furthermore, the cap strips feature very good transparency with transmission values exceeding 90%. This further enables their use in low volume real-time PCR

applications, which traditionally show decreased fluorescence signals. Taken together, these features may lead to improved sensitivity of real-time PCR experiments, which is especially advantageous for detection of samples with low nucleic acid concentrations.

Introduction

In PCR applications low volume PCR reactions have become more common due to the continuous increase in sample throughput. This approach offers the advantage of reduced reagent costs. The increased numbers of samples, but also the reduced reaction volumes, often require an automated PCR setup, since this allows for precise dispensing of even the smallest volumes. For these applications, the consumables used play a critical role. Low profile tubes can be used; however, the disadvantage remains that these tubes fit exclusively into specifically modified thermo blocks of PCR machines. In contrast, in standard 0.2 mL tubes or plates, respectively, the vapor volume is very high, which may lead to undesired condensation along the vessel walls or lids when volumes below 10 µL are used.

This will result in changes regarding the concentration of reaction components, which, in turn, may lead to reduced PCR efficiency or, in the worst case, to PCR failure. It can be shown here that by sealing with the Eppendorf Masterclear Cap Strips the vapor volume of standard 0.2 mL tubes can be noticeably reduced due to the inverted dome. Thus, the Eppendorf Masterclear Cap Strips are ideally suited for use with low volume PCR reactions. Furthermore, the optical window, which displays transmission values >90 %, enables use with low volume real-time PCR, which is often accompanied by low intensity fluorescence signals

Materials and Methods

Transmission measurements of the Masterclear Cap Strips as well as of cap strips by two competing manufacturers were performed in a Sapphire2™ (Tecan). For this purpose, the 8 caps of each strip were measured individually, using a wavelength spectrum of 400 to 750 nm in 50 nm intervals. The values were subsequently averaged, and the standard deviations were calculated.

For the real-time PCR experiments, the following PCR system was used in a SYBR Green application:

- > PCR target: 108 bp fragment of lambda DNA
- > Forward primer (600 nM): cgcacaggaactgaagaatg,
- > Reverse primer (300 nM): ccgtcgagaatactggcaat,
- > Template: lambda DNA (Roche)

A serial dilution of lambda DNA was prepared manually, resulting in a range of 100 – 1x10⁸ copies per reaction. In order to exclude the possible influence of pipetting inaccuracies, all further components were added to the different DNA concentrations. 12 replicates of 2.5 µL each of these mini master mixes were pipetted into the respective wells of a twin.tec real-time PCR Plate (semi-skirted), using the Eppendorf epMotion® 5070.

Subsequently, 3 columns of the plate were sealed with the Eppendorf Masterclear Cap Strips, while the remaining columns were sealed with 3 cap strips each by other manufacturers.

The plate was then centrifuged at 500 x g for 1 min, and a real-time PCR was performed in the Mastercycler® ep realplex4 S using the following program:

Initial Denaturation	Denaturation	Annealing Elongation
95 °C	95 °C	60 °C
2 min	10 s	30 s
40 Cycles		

Results and Discussion

Flat or domed lids are often used for sealing tubes and plates during PCR and real-time PCR. In contrast, the Eppendorf Masterclear Cap Strips feature an inverted dome, which reduces the vapor pressure volume of a tube (Figure 1).

the especially thin-walled optical windows of the Masterclear Cap Strips feature very good transparency for light of the wavelengths between 450 and 700 nm (Figure 2).

Figure 1:

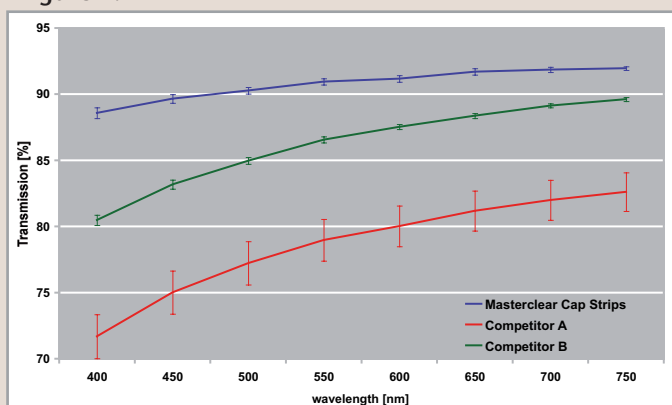


Vapor volume of 0.2 ml PCR tubes with different tube lids

On the right, the vapor volume resulting from the use of a flat lid (dark gray) is shown. Using the Masterclear Cap Strips (light gray), the vapor volume is reduced.

Thus, the total volume of an empty 0.2 mL PCR tube is reduced by 44 % as compared to a flat lid. The resulting reduction of vapor pressure allows for small volume PCR reactions to be performed in standard PCR tubes and plates. Transmission values exceeding 90 % are achieved in particular for those wavelengths above 500 nm, which are relevant to real-time PCR. In contrast, the competitors' strips are less transparent. Furthermore, it becomes evident that the transparency of the cap strips is largely dependent on the wavelength, i.e. the transmission rate of Competitor B's cap strips ranges between 80 % at 400 nm and a maximum of

Figure 2:



Transmission rates of different cap strips

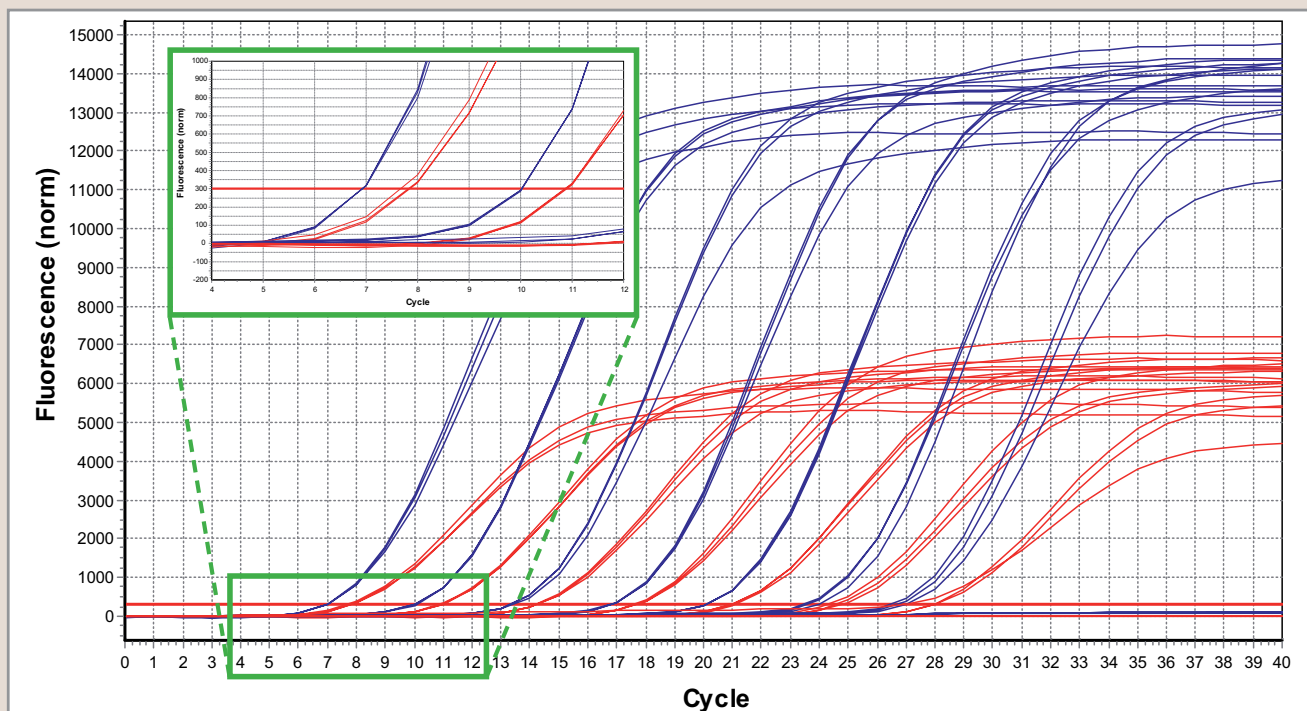
The transmission values of 8 individual lids of one strip were measured across a range of 450 – 700 nm wavelengths. The values were averaged, and the standard deviations are expressed as error bars.

89 % for the longer wavelengths. In the case of Competitor A, this dependence on wavelength is especially dominant; however, the transmission rates are noticeably lower: in the short wavelength range values of around 71 % are achieved, reaching a maximum of 82 % at 700 nm. In addition, the individual windows of the Eppendorf Masterclear Cap Strips feature low variance: the standard deviation across the range of 450-700 nm averages around 0.3. Competitor B's cap strips are comparable, whereas Competitor A's cap strips show a median standard deviation of 1.6.

During real-time PCR, most instruments measure fluorescence through the container lid. This should therefore be as thin as possible in order to maximize excitation of fluorescence as well as ensure maximum fluorescence signals during detection. Apart from this, optical windows should show low variability to ensure high reproducibility during measurements of replicates.

Thus be distinguished from background noise at an earlier time point. This improvement leads to an earlier detection of all nucleic acid dilutions by approximately one PCR cycle (see small insert in figure 3), thus improving assay sensitivity by a factor of nearly 2 in the case of 100 % amplification efficiency. This advantage may be crucial during analysis of samples with low nucleic acid concentrations.

Figure 3:



Low volume real-time PCR using different cap strips

Dilutions of 100 to 1×10^8 copies of lambda DNA per reaction mix (2.5 μL) were amplified in the presence of SYBR Green in a twin.tec real-time PCR Plate, which was sealed with Masterclear Cap Strips (blue) and cap strips from Competitor A (red).

The real-time PCR experiment shows that due to the high transparency of the Masterclear Cap Strips high enough fluorescence signals could be measured for a reaction volume as small as 2.5 μL . In addition, the signals measured through Eppendorf Masterclear Cap Strips were twice as high as those measured through cap strips from Competitor A (Figure 3). The increase of the fluorescence signal can

Conclusion

The Eppendorf Masterclear Cap Strips make extremely low volume PCR reactions possible, because their inverted domes reduces the vapor volume of PCR tubes and plates. Furthermore, the thin optical windows enhance the sensitivity of real-time PCR experiments.

These advantages become evident in real-time PCR systems with low fluorescence values and during detection of nucleic acid samples of low copy number.

Ordering information

Description	Order no. international	Order no. North America
Masterclear Cap Strips, 10 x 12	0030 132.874	951022089
real-time PCR Tube Strips without caps, 10 x 12	0030 132.882	951022102
Masterclear Cap Strips and real-time PCR Tube Strips, 10 x 12 each	0030 132.890	951022109
Eppendorf twin.tec® 96 real-time PCR Plate, skirted, white frame	0030 132.513	951022015
Eppendorf twin.tec® 96 real-time PCR Plate, skirted, blue frame	0030 132.505	951022003
Eppendorf twin.tec® 96 real-time PCR Plate, semi-skirted, white frame	0030 132.548	951022055
Eppendorf twin.tec® 96 real-time PCR Plate, semi-skirted, blue frame	0030 132.530	951022043
Eppendorf twin.tec® 96 real-time PCR Plate, unskirted, white frame	0030 132.700	0030 132.700
Eppendorf twin.tec® 96 real-time PCR Plate, unskirted, blue frame	0030 132.718	0030 132.718
Eppendorf twin.tec® 96 real-time PCR Plate, semi-skirted, forensic DNA grade	0030 129.644	0030 129.644
Eppendorf twin.tec® 96 real-time PCR Plate, skirted, forensic DNA grade	0030 129.636	0030 129.636
epMotion® 5070, Automated pipetting system	5070 000.000	960000005

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