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Absence of PCR Inhibitory Effect in Eppendorf Filter Tips

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Abstract

Filter tips are commonly used in PCR application to avoid sample cross-contamination, which is one of the major issues encountered. Another major concern is regarding inhibition of the PCR reaction. During pipetting, it may be possible that the sample liquid comes into contact with the filter due to improper handling or accidental over-pipetting. In this case, it is important to exclude PCR inhibition due to contact with the filter. The study presented herein shows that Eppendorf filter tips do not cause any PCR inhibitory effect even if the sample may come into contact with the filter materials, and hence they are highly suitable for use in PCR application.

Introduction

Polymerase chain reaction (PCR) has become an indispensable application in the field of molecular biology. Being a sensitive method, special precautions are employed to avoid cross-contamination. However, cross-contamination is not the only factor in consideration for successful PCR and concurrently reliable results. There are situations where expected amplification products are not obtained or false negative results are unknowingly being taken as true results, leading to incorrect analysis. Failure to amplify can be partly attributable to the presence of PCR inhibitors in the reaction.

Sources of PCR inhibitors such as blood and certain tissue samples as well as the reagents used for sample processing and nucleic acid purification are commonly known. On the other hand, inhibitors originating from the consumables used in the PCR workflow such as tips, tubes and plates are the least expected or even totally disregarded. The latter cannot be completely ruled out. Potential PCR inhibitors may be introduced during the manufacturing process or they are inherent in the materials used to make the consumable.

Filter tips are recommended to be used preferably in all steps along the PCR workflow to minimize the risk of cross-contamination by aerosols during pipetting. There exists another type of filter tip, generally referred to as self-sealing filter tip, which provides additional protection in case of accidental over-pipetting by forming a barrier against the incoming liquid. This is made possible by including a layer of swellable substance in addition to the filtering material. Regardless of the filter tip type and in any case where the sample liquid comes into contact with the filter, there must be no PCR inhibitory effect caused by the filter materials.

Eppendorf ep Dualfilter T.I.P.S.® and ep Dualfilter T.I.P.S.® SealMax have two filter phases of well-defined pore sizes to effectively guard against aerosol contamination. The self-sealing filter tip, ep Dualfilter T.I.P.S. SealMax, additionally forms a reliable barrier upon contact with liquid, thus preventing it from passing through the filter and contaminating the pipette. The following experiment shows that both ep Dualfilter T.I.P.S. as well as ep Dualfilter T.I.P.S. SealMax do not cause any PCR inhibitory effect in case the sample liquid comes into temporary contact with the filter materials.

Materials and Methods

Real-time PCR method was used to detect potential PCR inhibitory effect.

ep Dualfilter T.I.P.S. and ep Dualfilter T.I.P.S. SealMax of size 200 µL were tested. For each tip type and each test condition described below, 5 tips were taken randomly from the tip rack for individual testing.

With the test tip attached, 300 µL of molecular biology grade water was aspirated with reverse pipetting technique using Eppendorf Research® plus 30–300 µL pipette. The sample water penetrated through the filter in ep Dualfilter T.I.P.S. whereas it was prevented from passing through the filter in ep Dualfilter T.I.P.S. SealMax. In both cases, the sample liquid was in contact with the filter material and was left as such for a time period of 1 minute, 2 minutes and 5 minutes respectively. After the elapsed time period, the sample liquid was recovered from the tip and subsequently used as water source for the PCR reaction setup.

The PCR reaction was set up to amplify a 146 bp fragment of the human GAPDH (glyceraldehyde 3-phosphate dehydrogenase) gene.

Forward primer: 5'-TGC CTT CTT GCC TCT TGT CT-3'
Reverse primer: 5'-GGC TCA CCA TGT AGC ACT CA-3'

Table 1 shows the composition of a single PCR reaction. A sufficient volume of mastermix containing all required components except water was first prepared and distributed equally. The water recovered from the test filter tip was then added, and mixed thoroughly, to complete the PCR reaction composition. Three replicates of positive control (using water source without contact with filter) and negative control (absence of human DNA) were included in the PCR run.

Table 1: Real-time PCR composition of a single reaction.

Component	Volume [µL]	Final concentration
KAPA SYBR® FAST Universal qPCR Master Mix (Kapa Biosystems)	5	1x
GAPDH primers	0.3 each	0.3 µM each
Human DNA	0.4	16 copies
Water recovered from test filter tip after over-pipetting	4	–

Real-time PCR was carried out on Eppendorf Mastercycler® ep **realplex** instrument with the following cycling conditions:

Pre-denaturation	95 °C for 2 minutes
40 cycles of:	
Denaturation	95 °C for 3 seconds
Extension	60 °C for 20 seconds

Results and Discussion

Real-time PCR was chosen over conventional PCR as a means to detect potential PCR inhibitory effect for the reason that it is a highly sensitive quantitative method. The occurrence of PCR inhibition, including the extent of inhibition, can be detected. This is possible by comparing the threshold cycle, Ct (intersection between amplification curve and threshold line), which is a relative measure of the target DNA concentration in the PCR reaction. Given the same starting amount of DNA template, changes in the Ct value will indicate that different amounts of PCR product are being produced due to some sort of inhibition on the PCR process. The extent of the Ct value change will, in turn, implicate the different levels of inhibition.

Figure 1 summarizes the average Ct values of the test filter tips (ep Dualfilter T.I.P.S. and ep Dualfilter T.I.P.S. SealMax) and positive control, while Figure 2 shows a representative amplification plot. In Figure 1, 1 min, 2 min and 5 min are referring to the incubation or contact time of the sample water with the filter materials of the test filter tips by over-pipetting. For positive control, there was no incubation involved but instead, the PCR reaction was prepared from sample water which had not been in contact with the filter materials. There is no significant shift in the average Ct values of the test filter tips in comparison to that of positive control. In fact, the difference of the Ct values between the test filter tips and positive control lies within the range of ± 1 cycle. This shows that contact of the sample liquid with the filter materials of Eppendorf filter tips in case of over-pipetting does not lead to adverse PCR inhibitory effect. In addition, the deviations among the individual test tips of one filter tip type are very low, as depicted by the small error bars. Therefore, one can highly rely on the filter performance (absence of PCR inhibitory effect) from one tip to the other.

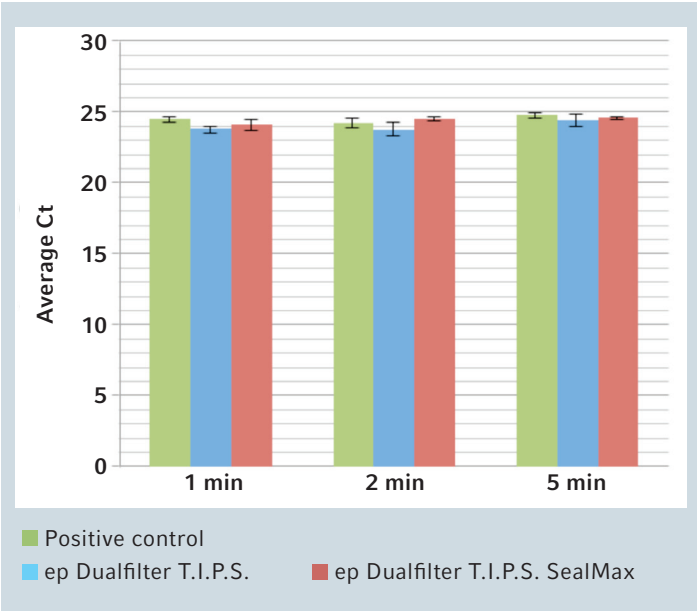

Fig. 1: Average Ct values of test filter tips (5 replicates of each) and positive control (3 replicates). The error bar represents standard deviation. 1 min, 2 min and 5 min refers to the contact time of the sample water with the filter materials of the test filter tips by over-pipetting, while positive control did not involve any contact with the filter materials.

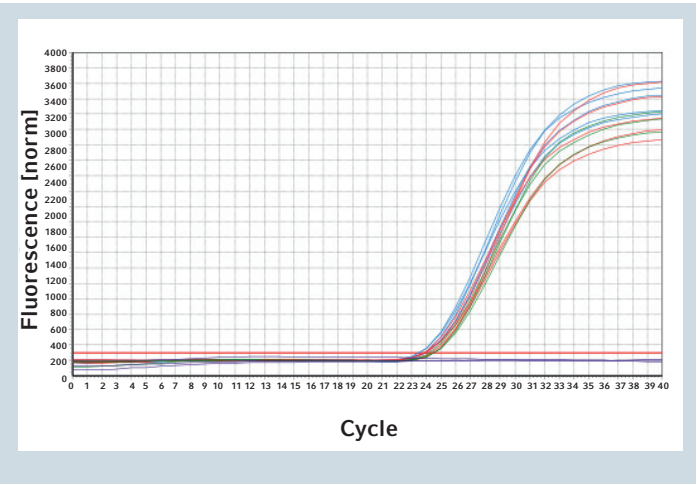

Fig. 2: Amplification plot for 1 min contact time of the sample water with the filter materials of the test filter tips. Respective amplification plot for 2 min and 5 min contact time is not shown. The amplification pattern is similar to that of 1 min contact time as depicted here.
Green curves: positive control (3 replicates)
Blue curves: ep Dualfilter T.I.P.S. (5 replicates)
Red curves: ep Dualfilter T.I.P.S. SealMax (5 replicates)

In practice, the sample liquid comes into contact with the filter during over-pipetting for a short time only. In this test, longer contact time up to 5 minutes was tested and still there was no PCR inhibitory effect observed. This could account for incidences whereby the sample may come into contact with the filter for longer times, for example, when user is unaware of the occurrence of over-pipetting.

Eppendorf filter tips are certified to be free of PCR inhibitors. Each batch is tested and certified by an independent accreditated laboratory to avoid any bias. The results of this experiment provide further evidence that the filter tip, including the filter materials, do not cause any PCR inhibitory effect. Samples can be safely used for further downstream processing.

Conclusion

Apart from providing effective protection against aerosol contamination, use of Eppendorf filter tips does not lead to any PCR inhibitory effect, even in cases where the sample liquid may come into contact with the filter materials during over-pipetting. Given the various sources of PCR inhibitors, using consumables such as filter tips that are certified to be free of PCR inhibitors will ensure security of the samples besides helping to eliminate one of the possible factors of PCR inhibition. Thus, Eppendorf filter tips are well-suited for use in PCR work.

Ordering information

Product	Int. Order no.	North America Order no.
ep Dualfilter T.I.P.S.® SealMax, Racks, PCR clean/Sterile, 10 × 96 tips		
0.1–10 µL S, 34 mm	0030 077.806	0030077806
0.5–20 µL L, 46 mm	0030 077.814	0030077814
2–100 µL, 53 mm	0030 077.822	0030077822
2–200 µL, 55 mm	0030 077.830	0030077830
20–300 µL, 55 mm	0030 077.849	0030077849
50–1,000 µL, 76 mm	0030 077.857	0030077857
ep Dualfilter T.I.P.S.®, Racks, PCR clean/Sterile, 10 x 96 tips		
0.1–10 µL S, 34 mm	0030 077.504	022491202
0.1–10 µL M, 40 mm	0030 077.512	022491211
0.5–20 µL L, 46 mm	0030 077.520	022491229
2–20 µL, 53 mm	0030 077.539	022491270
2–100 µL, 53 mm	0030 077.547	022491237
2–200 µL, 55 mm	0030 077.555	022491296
20–300 µL, 55 mm	0030 077.563	022491245
50–1,000 µL, 76 mm	0030 077.571	022491253
ep Dualfilter T.I.P.S.®, Racks, PCR clean/Sterile, 5 x 48 tips		
50–1,250 µL L, 103 mm	0030 077.750	022494002
0.1–5 mL, 120 mm	0030 077.580	022491261
1–10 mL L, 243 mm, individually blistered, 50 pieces per package	0030 077.598	022491288

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