Sartorius Pipette Tips are Free from PCR Inhibitors

Sartorius pipette tips have been tested to be free from PCR inhibitors by real-time quantitative PCR amplification. For tips to be certified as PCR-inhibitor free, the threshold cycle (Ct) value difference compared to the negative control should not exceed 2 cycles.

Test Method for PCR Inhibitors

The Sartorius Microsart® ATMP Mycoplasma Detection Kit (Order No. SMB95-1003|1004) was utilized to assess whether pipette tips leach potential contaminants that may inhibit PCR reactions. Each sample was prepared as follows: 50 pipette tips were rinsed once with 50 µL of PCR-grade water, or the maximum volume of the tip if it was less than 50 µL. The total sample volume was 100 µL.

Triplicate samples were prepared for each product. This procedure aims to accumulate any contaminants leaching from the tips. The reaction mix was prepared in accordance with the kit's manual. Subsequently, 10 µL of the sample was pipetted to 15 µL of the reaction mix, which contained internal control DNA. PCR-grade water served as the negative control. The PCR was conducted for 45 cycles, monitoring the ROX signal of the internal amplification control. A difference of ≥2 in the Ct value between sample and control was considered indicative of inhibition. None of the tested pipette tips (Table 1) had any inhibitory effect on the PCR reaction. Based on this one-time analysis conducted, all products tested were negative for PCR inhibition. It is important to emphasize that this result is indicative only of the specific lots tested during this analysis.

Table 1: *The following Sartorius pipette tips were tested for PCR inhibitors. Tips are certified as PCR inhibitor free if the ΔCt value does not exceed 2. A difference of ≥2 in the average Ct value of the sample triplicates compared to the negative control was not identified for any of the products. The standard deviations (SD) for the Ct values of the triplicate samples are also shown in the table.*

Item no.	Product description	ΔCt compared to negative control (PCR-grade water)	Ct SD
790010	Optifit Tips Single Tray 10 µL	-0.192	0.064
790200	Optifit Tips Single Tray 200 µL	-0.214	0.164
790202	Optifit Tips Refill Tower 200 µL	-0.002	0.119
791000	Optifit Tips Single Tray 1,000 µL	-0.010	0.112
791002	Optifit Tips Refill 1,000 µL	-0.241	0.159
790021F	Safetyspace Filter Tip Single Tray 20 µL Pre-sterilized	-0.190	0.082
LH-B790204	Optifit Tips FlexiBulk 200 µL	-0.117	0.134
790101F	Safetyspace Filter Tip Single Tray 120 µL Pre-sterilized	-0.029	0.278
790350	Optifit Tips Single Tray 350 µL	-0.046	0.136
790301F	Safetyspace Filter Tip Single Tray 300 µL Pre-sterilized	-0.129	0.246
790201F	Safetyspace Filter Tip Single Tray 200 µL Pre-sterilized	-0.095	0.244
790011F	Safetyspace Filter Tip Single Tray 10 µL Pre-sterilized	0.067	0.061
790203	Optifit Tips Refill 200 µL Pre-sterilized	-0.010	0.208
790352	Optifit Tips Refill Tower 350 µL	-0.135	0.039
LH-X791000	Optifit Tips Single Tray 1,000 µL Extended	0.005	0.257

Positive Control for PCR Inhibition

High concentrations of calcium may affect the availability of magnesium as a cofactor for the DNA polymerase, leading to decreased polymerase activity [1,2]. An 1M $CaCl_2$ solution served as positive control for PCR inhibition, and a dilution series of this stock solution was prepared to evaluate the concentrations at which PCR inhibition occurred. The PCR was conducted for 45 cycles, monitoring the ROX signal of the internal amplification control. The dilution curve of the solution and the Ct values are presented in Table 2. The Ca^{2+} concentration in Sample 1 (40,000 µM) and 2 (4,000 µM) clearly inhibited the PCR reaction (ΔCt ≥2).

Table 2: *Ca^{2+} is a PCR inhibitor. A serial dilution of a $CaCl_2$ solution was analyzed on the QuantStudio™ 5 Real-Time PCR System (Applied Biosystems), applying the Sartorius Microsart® ATMP Mycoplasma Detection Kit. Triplicate samples were analyzed, and the average Ct values for the internal control were calculated. The difference between the samples and the negative control were calculated.*

Sample	$CaCl_2$ concentration in final PCR sample (µM)	Average Ct	ΔCt
1	40,000	45	11.725
2	4,000	42.334	9.059
3	400	33.015	-0.26
4	40	33.077	-0.198
5	4	33.437	0.162
6	0.4	33.28	0.005
7	0.04	33.439	0.164
Negative control	0	33.275	

References

1. Sidstedt M, Rådström P, Hedman J. PCR inhibition in qPCR, dPCR and MPS-mechanisms and solutions. Anal Bioanal Chem. 2020 Apr;412(9):2009-2023. doi: 10.1007/s00216-020-02490-2. Epub 2020 Feb 12. PMID: 32052066; PMCID: PMC7072044.
2. Bickley J, Short JK, McDowell DG, Parkes HC. Polymerase chain reaction (PCR) detection of Listeria monocytogenes in diluted milk and reversal of PCR inhibition caused by calcium ions. Lett Appl Microbiol. 1996 Feb;22(2):153-8. doi: 10.1111/j.1472-765x.1996.tb01131.x. PMID: 8936376.

Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Straße 20
37079 Göttingen
Phone +49 551 308 0

USA
Sartorius Corporation
3874 Research Park Drive
Ann Arbor, MI 48108
Phone +1 734 769 1600

For further information, visit
sartorius.com