

## Delta Filter™ Technology

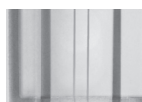
*Security and protection with industry's first aerosol barrier pipette tip with color-change visual alert*

### INTRODUCTION

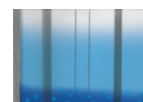
Within the past two decades, the use of aerosol barrier pipette tips, also referred to as “filter tips”, has become a best practice in protocols utilizing PCR amplification<sup>1</sup>. Moreover, the efficiency of filter tips in preventing cross contamination of micro-particles has made it a standard consumable cited in instruction manuals for laboratories performing molecular assays in research<sup>2</sup> and in the clinic<sup>3,4</sup>. Though generally highly effective, errors in pipetting such as overdraws and splash, can challenge even the most robust filter. In those instances, it is impossible to visually detect when exposure to aerosols or liquids has occurred with standard aerosol barrier tips. Failure to quickly verify filter efficacy can lead to costly contamination issues including failed assays, tedious clean-up of pre-PCR workspaces and, at worst, shut down of operations.

Biotix has introduced a line of aerosol barrier pipette tips which addresses this concern with a proprietary technology called Delta Filter™. This technology incorporates a chemically inert, color change compound which not only traps aerosols and absorbs liquids, but turns blue

upon contact with contaminants. This feature safeguards critical assays with instant visual detection, and also helps to identify technician pipetting errors and reagent issues such as foaming.



Delta Filter



Delta Filter Activated

In an extensive comparison against the leading brands on the market, Biotix tips with Delta Filter technology proved to be highly effective in blocking aerosols and appropriate for use with even the most sensitive biological samples. This technical bulletin provides an overview of data generated from a comprehensive study conducted by MRIGlobal, a leading national research institute. The study design consisted of two parts: a rigorous cross-brand analysis of aerosol blocking efficiency down to nanoparticle levels, and an inhibition and enhancement study of Delta Filter.

**Table 1: Aerosol Barrier Tips Used in Study**

Manufacturer	Part Number	Lot Number	Description
Biotix	M-0100-9FC	0309 65464	100 µl Filter Pipette Tip, Sterile
Molecular BioProducts (MBP)	2065	09440809	ART™ 100 µl Filter Pipette Tip, Sterile
Axygen Scientific	TF-100-L-R-S	302-28-151	100 µl MAXYmum Recovery Filter Tip, Sterile

**AEROSOL BLOCKING EFFICIENCY**

Filter tips from Biotix and two leading brands on the market were evaluated for effectiveness in blocking aerosols. Aerosol blockade was tested on solutions containing PCR amplicon, bacterial culture (*E. coli*), vaccinia viral particles, and nanoceria (30nm CeO<sub>3</sub>). Amplicon contamination was detected utilizing realtime PCR, bacteria by plate counts, viral particle by realtime PCR, and nanoceria by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Samples were processed in triplicate and analyzed in duplicate.

**PCR Amplicon Challenge**

**Methods**

A previously generated amplicon of 176 bp at a concentration of 24 ng/ml was utilized for testing filter efficiency in blocking PCR aerosols. Samples were prepared by pipetting 100 µl of the solution up and down with a 200 µl pipettor three full times, with a final dispense back into the original tube. The tips were carefully removed, and with a

new pipettor and fresh tip, the inside of the used tip above the filter was washed by pipetting and dispensing 3 times with 100 µl of nuclease-free dH<sub>2</sub>O. The wash was then moved to a fresh 0.5 ml tube. This was repeated in triplicate for each of the three pipette tip brands. Ten microliters of the resulting samples were analyzed in duplicate by realtime PCR using standard cycling conditions for a total of 50 cycles on a BioRad iCycler. Positive controls were the starting amplicon at 1:10 and 1:100 dilutions. Negative controls were no template controls with molecular grade water.

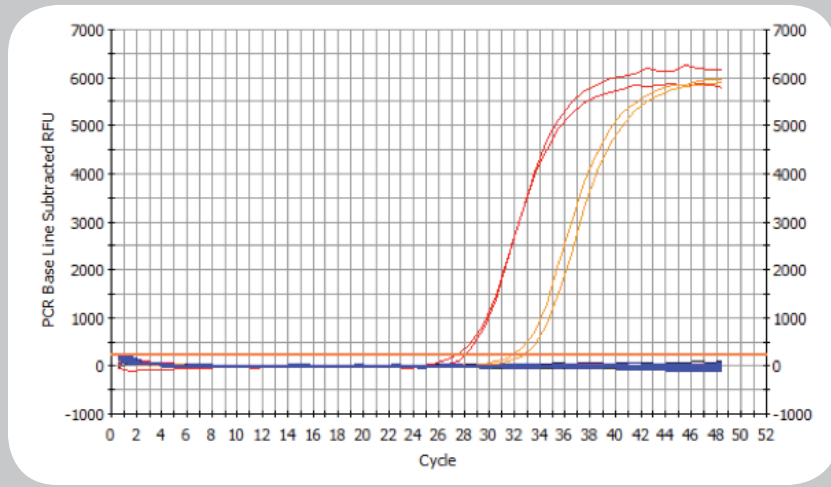
**Results**

Targeted amplification showed no amplified product for all three brands, indicating equal efficiency in blocking PCR amplicon aerosols (Table 2, Figure 2). Positive controls showed expected amplification and negative controls showed no amplification.

**Table 2: PCR for Amplicon Aerosols**

Sample	Replicates	Mean Ct	Ct SD
Positive Control - Amplicon 1:10	3	27.61	0.563
Positive Control - Amplicon 1:100	3	32.22	0.581
Biotix Tip – Amplicon Wash	6	N/A	N/A
Axygen Tip – Amplicon Wash	6	N/A	N/A
MBP ART Tip – Amplicon Wash	6	N/A	N/A
Negative Control	2	N/A	N/A

**Figure 2: Amplification for Amplicon Aerosols**



**Bacterial Challenge**

**Methods**

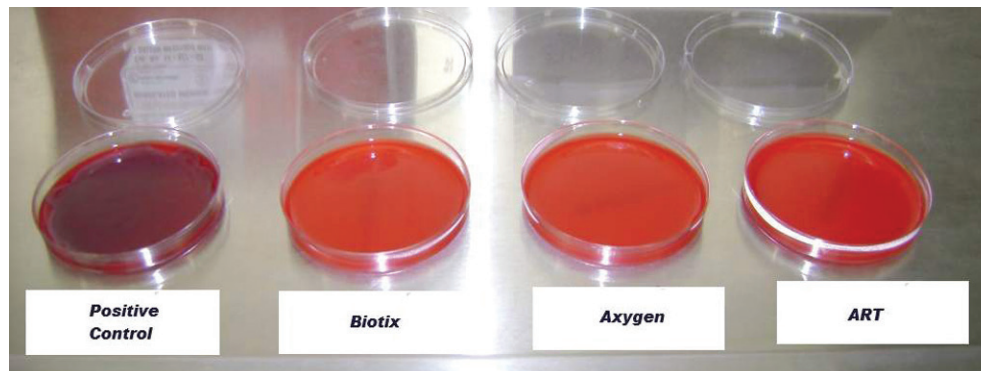
A culture of *Escherichia coli* was grown in BHI broth to an OD of 1.0. Samples were prepared by pipetting 100 µl of the culture up and down with a 200 µl pipettor three full times, with a final dispense back into the original tube. The tips were carefully removed, and with a new pipettor and fresh tip, the inside of the used tip above the filter was washed by pipetting and dispensing three times with 100 µl of sterile BHI broth. The broth was moved to a fresh 0.5 ml tube. This was repeated in triplicate for each of the three pipette tip brands. Analysis was performed

by plating 100 µl of sample in duplicate on BHI plates. Plates were incubated for 24 hours at 37°C, with bacterial colony counts taken after.

**Results**

Representative plates are shown in Figure 3. Control plates with the original starting *E.coli* stock show a lawn of growth, whereas plates containing washes from above the filters of all three tip brands showed no bacterial growth. This indicates equal effectiveness in blocking bacterial aerosols. No growth was observed even after 96 hours of incubation.

**Figure 3: Bacterial Growth at 24 Hours**



**Viral Challenge**

**Methods**

Culture media containing  $1 \times 10^8$  pfu/ml vaccinia virus was utilized to test filter blocking efficiency of viral aerosols. Samples were prepared by using each brand of tip to draw 100  $\mu$ l of the solution up and down with a 200  $\mu$ l pipettor three full times, with a final dispense back into the original tube. The tips were carefully removed, and with a new pipettor and fresh tip, the inside of the used tip above the filter was washed by pipetting and dispensing 3 times with 100  $\mu$ l of sterile PBS. The PBS wash was then transferred to a new 0.5 ml tube. This was repeated in triplicate for each of the three brands of tips. DNA was isolated from each resulting sample. The starting viral stock and 10  $\mu$ l of the isolated viral DNA samples were analyzed in duplicate by

realtime PCR with standard cycling conditions for a total of 50 cycles on a BioRad iCycler. Positive controls were the starting viral stock at 1:10 through 1:10,000 dilutions. Negative controls were no template controls with molecular grade water and no virus control through extraction.

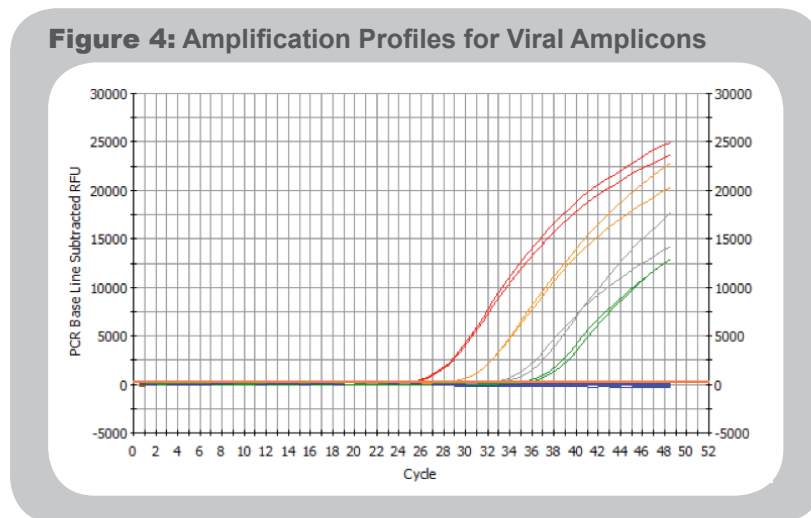
**Results**

Following viral DNA isolation, targeted amplification of the virus that potentially crossed the filter barrier of the pipette tips showed no amplifications for all three brands (Table 3 and Figure 4). Each tip demonstrated equal efficiency in blocking viral aerosols. Positive controls amplified as expected, and Negative controls had no amplification.

**Table 3: PCR for Viral Amplicon Aerosols**

Sample	Replicates	Mean Ct	Ct SD
Positive Control - Virus 1:10	2	24.92	0.004
Positive Control - Virus 1:100	2	28.23	0.035
Positive Control - Virus 1:1000	2	32.60	0.448
Positive Control - Virus 1:10000	2	34.92	0.486
Biotix Tip Wash	6	N/A	N/A
Axygen Tip Wash	6	N/A	N/A
MBP ART Tip Wash	6	N/A	N/A
Negative control: No template	2	N/A	N/A

**Figure 4: Amplification Profiles for Viral Amplicons**



**Nanoparticle Aerosol Challenge**

**Methods**

A solution containing 82,000 ng/ml CeO<sup>3</sup> was used for testing filter blocking efficiency against nanoparticle aerosols. Samples were prepared by pipetting 100 µl of the solution up and down with a 200 µl pipettor three full times with final dispense back into the original tube. The tips were carefully removed, and with a new pipettor and fresh tip, the inside of each used tip above the filter was washed by pipetting and dispensing three times with 100 µl of sterile dH<sub>2</sub>O. The wash was then moved to a fresh 0.5 ml tube. This process was repeated in triplicate for each brand of pipette tip. Detection

for the presence of CeO<sup>3</sup> was performed on an Agilent 7500 CE Inductively Coupled Plasma Mass Spectrometer (ICP-MS) with standard assay conditions. Positive controls were the starting CeO<sup>3</sup> stock at 82,000 ng/ml. Negative controls were sterile water.

**Results**

Analysis of washes for aerosols containing cerium nanoparticles showed no breakthrough of aerosols across the filter barriers for all three pipette tip brands (Table 4). Positive and negative controls produced the expected results.

**Table 4: ICP-MS Detection of Aerosols of Cerium Nanoparticles**

Sample	Elements	Mass	Replicates	Mean Concentration (ng/ml)	SD	Range
Positive Control	Ce	140	2	73545.5	8703.777	67391-79700
Biotix	Ce	140	3	>0.019	N/A	N/A
Axygen	Ce	140	3	>0.019	N/A	N/A
MBP ART	Ce	140	3	>0.019	N/A	N/A
Negative Control	Ce	140	2	>0.019	N/A	N/A

**DELTA FILTER INHIBITION AND ENHANCEMENT TESTING**

As a new technology innovation and a singular product of its kind on the market, it was important for Biotix to verify the suitability of Delta Filter for use in biological and molecular assays. Of key focus was confirming that the color change compound was chemically inert, and would neither inhibit nor enhance the most sensitive biological assays. To test this, colorant was eluted from the Delta Filter into molecular grade water (MGW) and used in realtime PCR to determine any PCR inhibition; eluted into whole blood and tested in PCR and ELISA applications; and eluted into cell culture media to test for toxicity. Biotix filter tips were compared against the competitive brands broadly used in the market.

**Molecular Grade Water and PCR Challenge**

**Methods**

The filters were removed from each brand of filter tip with sterile forceps and placed into a 1.5 ml tube with 1.0 ml of molecular grade water. Filters were incubated for two hours at room temperature to allow any extractable to elute into the water. A series of dilutions were made consisting of:

- (1) 200 µl and placed in a fresh tube
- (2) 100 µl in fresh tube with 100 µl MGW
- (3) 50 µl in fresh tube with 150 µl MGW
- (4) 25 µl in fresh tube with 175 µl MGW
- (5) A control tube with 200 µl MGW

PCR inhibition was measured by substituting the eluted water for the molecular grade water normally used in the set-up of realtime PCR reactions. The template used was normal human DNA isolated from whole blood and amplified with primer and probes for human RNaseP. Eight microliters of each elution sample was analyzed in duplicate by realtime PCR with standard cycling conditions for a total of 50 cycles on a BioRad iCycler. Positive controls were the starting human whole blood DNA at 1:10 to 1:10,000 dilutions. Negative controls were no template controls and an extraction control.

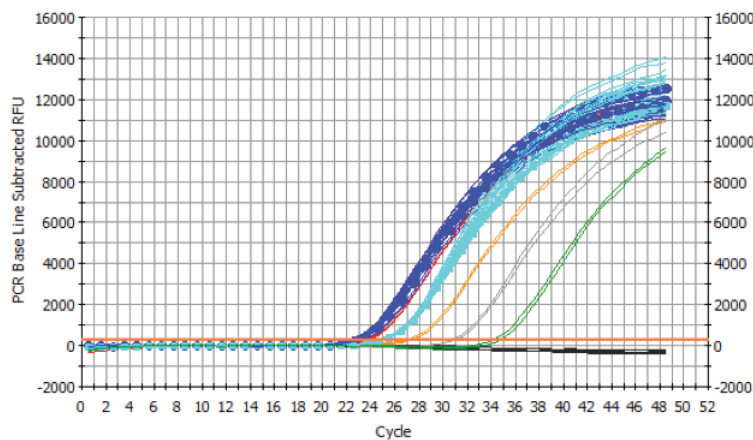
**Results**

There was no observed inhibition of PCR by eluates in molecular biology grade water from any of the filters from the three different brands of tips as compared to controls (Table 5 and Figure 5). A serial dilution of the eluates was performed, but as no dose response to the eluates was seen, data were combined and treated as replicates. All three tip brands performed equally well, with no difference in amplification as compared to each other and controls.

**Table 5: PCR Inhibition Testing by Filter Eluate**

Sample	Replicates	Mean Ct	Ct SD
Positive Control - 1:10 WB DNA	2	23.62	0.197
Positive Control - 1:100 WB DNA	2	27.29	0.153
Positive Control - 1:1000 WB DNA	2	30.91	0.198
Positive Control - 1:10000 WB DNA	2	34.44	0.382
Biotix Tip	8	25.00	0.157
Axygen Tip	8	25.10	0.077
MBP ART Tip	8	25.13	0.143
Negative control – MGW	2	25.27	0.094
No Template Control – MM	2	N/A	N/A
No Template Control – SA	2	N/A	N/A

**Figure 5: Amplification Profiles for PCR Inhibition Test**



**Whole Blood and PCR Challenge**

**Methods**

Filters from each brand of tip were removed with sterile forceps and placed into a 1.5 ml tube with 1.0 ml of human whole blood (WB). Filters were incubated for two hours at room temperature to allow any extractable to elute into the blood. A series of dilutions were made consisting of:

- (1) 200 µl and placed in a fresh tube
- (2) 100 µl in fresh tube with 100 µl WB
- (3) 50 µl in fresh tube with 150 µl WB
- (4) 25 µl in fresh tube with 175 µl WB
- (5) A control tube with 200 µl WB

DNA was isolated from each blood sample and PCR inhibition was measured by realtime PCR. The template used was human DNA isolated from the whole blood samples and amplified with primer and probes for human RNaseP.

Ten microliters of DNA from each elution sample was analyzed in duplicate by realtime PCR with standard cycling conditions for a total of 50 cycles on a BioRad iCycler. Positive controls were the starting human whole blood DNA at 1:10 to 1:10,000 dilutions. Negative controls were no template controls with molecular grade water and extraction controls.

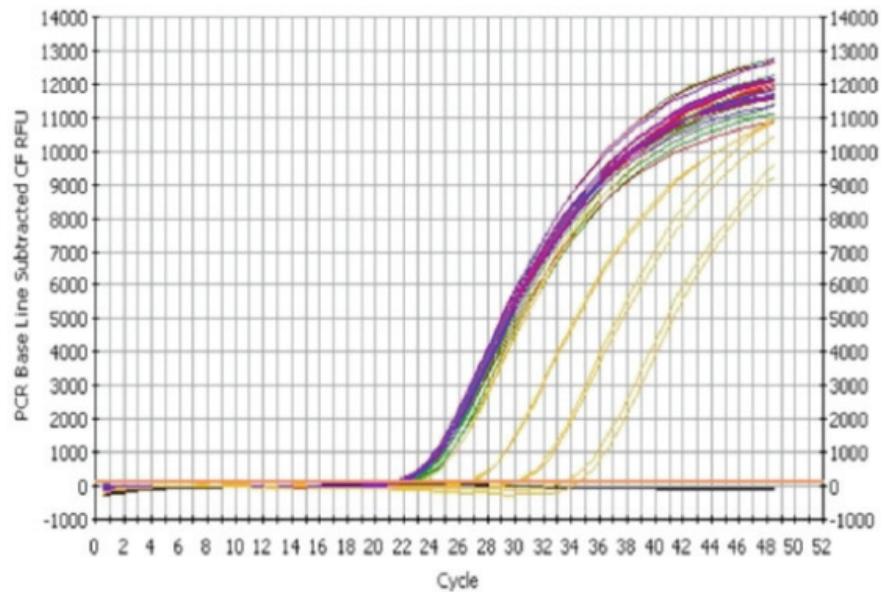
**Results**

There was no observed inhibition of PCR by eluates in whole blood from any of the filters from the three brands of tips as compared to controls (Table 6 and Figure 6). A serial dilution of the eluates was performed, but as no dose response to the eluates was seen, data were combined and treated as replicates. Biotix Delta Filter performed equally with no difference in amplification of human RNaseP as compared to the other tip brands and controls.

**Table 6: PCR Inhibition by Filter Elutes for Whole Blood**

Sample	Replicates	Mean Ct	Ct SD
Positive Control - 1:10 WB DNA	2	23.62	0.197
Positive Control - 1:100 WB DNA	2	27.29	0.153
Positive Control - 1:1000 WB DNA	2	30.91	0.198
Positive Control - 1:10000 WB DNA	2	34.44	0.382
Biotix Tip	8	22.98	0.115
Axygen Tip	8	22.56	0.019
MBP ART Tip	8	22.98	0.169
Negative control – MGW	2	22.98	0.039
No Template Control – MM	2	N/A	N/A
No Template Control – SA	2	N/A	N/A

**Figure 6: Amplification Profiles for PCR Inhibition**



**Methods**

The filters from samples of each pipette tip brand were removed with sterile forceps and placed into a 1.5 ml tube with 1.0 ml of human whole blood (WB). Filters were incubated for two hours at room temperature to allow any extractable to elute into the blood. A series of dilutions were made consisting of:

- (1) 200  $\mu$ l and placed in a fresh tube
- (2) 100  $\mu$ l in a fresh tube with 100  $\mu$ l WB
- (3) 50  $\mu$ l in a fresh tube with 150  $\mu$ l WB
- (4) 25  $\mu$ l in a fresh tube with 175  $\mu$ l WB
- (5) A control tube with 200  $\mu$ l WB

Fifty microliters of each eluted whole blood sample was analyzed for IgG2 by ELISA utilizing an Invitrogen Human IgG Subclass ELISA

Kit (Cat # 99-1000). Positive controls were the starting human whole blood. Negative controls were no sample addition/PBS.

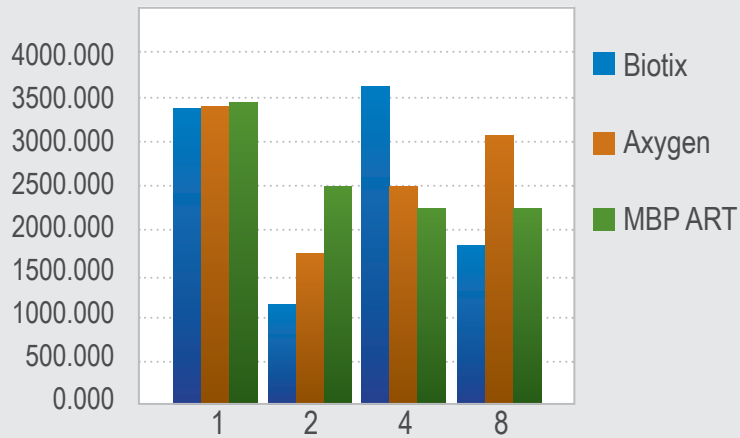
**Results**

There was no inhibition observed by eluates in whole blood of an ELISA for human IgG2 from any of the filter tips compared to controls (Table 7 and Figure 7). A serial dilution of the eluates was performed, but there was no dose response observed. Delta Filter performed equally well with no difference in detection of human IgG2 as compared to the other tips and controls.

**Table 7: ELISA Inhibition by Filter Eluates**

Sample	Replicates	Mean OD	SD	IgG2 (µg/ml)
Biotix – 1	2	0.986	0.029	3375.520
Biotix – 1:1	2	0.680	0.017	1165.336
Biotix – 1:4	2	1.003	0.037	3581.320
Biotix – 1:8	2	0.806	0.013	1803.833
Axygen – 1	2	0.989	0.046	3410.958
Axygen – 1:1	2	0.798	0.042	1754.288
Axygen – 1:4	2	0.897	0.050	2476.169
Axygen – 1:8	2	0.957	0.095	3051.372
MBP ART – 1	2	0.992	0.008	3452.773
MBP ART – 1:1	2	0.898	0.002	2484.805
MBP ART – 1:4	2	0.867	0.042	2230.606
MBP ART – 1:8	2	0.870	0.004	2254.025
Positive Control	2	0.912	0.034	2613.455

**Figure 7: IgG2 Profiles for ELISA Inhibition**



**Cell Culture Media and Toxicity Challenge**

**Methods**

The filters from samples of each tip brand were removed with sterile forceps and placed into a 1.5 ml tube with 1.0 ml of tissue culture media. Filters were incubated for two hours at room temperature to allow any extractable to elute into the culture media (CM). A series of dilutions were made consisting of:

- (1) 200 µl and placed in a fresh tube
- (2) 100 µl in a fresh tube with 100 µl CM
- (3) 50 µl in a fresh tube with 150 µl CM
- (4) 25 µl in a fresh tube with 175 µl CM
- (5) A control tube with 200 µl CM.

One hundred microliters of each elution culture media sample was added to monolayers of BSC-40 Green Monkey Kidney Cells plated at  $3.94 \times 10^3$  cells/well in 96-well plates. Cells were allowed to incubate in the elution cul-

ture media for 18 hours at 37°C. Media was replaced and MTT conversion was measured to assess cell viability as per instructions from the Invitrogen Vybrant MTT Cell Proliferation Kit (Cat #V-13154). The positive control was complete culture media. Negative control was no sample addition/PBS.

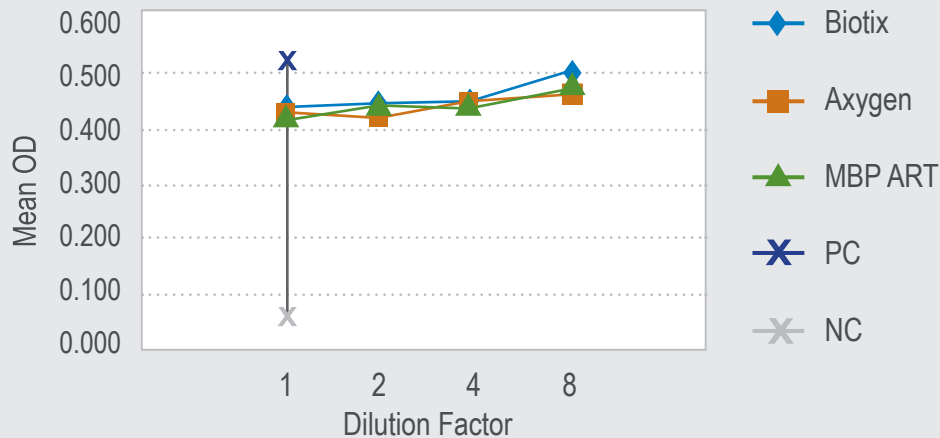
**Results**

There was no observed inhibition of cell viability as determined by MTT conversion by eluates in cell culture media from any of the filter tips tested compared to each other (Table 8 and Figure 8). A serial dilution of the eluates was performed and there was an observed linear dose response to the eluates with a difference of 0.5 to 1 OD unit from least to most concentrated as compared to controls. Delta Filter performed equally with no difference in cell toxicity as compared to the other brands.

**Table 8: Cell Toxicity of Filter Eluates in Culture Media**

Sample	Replicates	Mean OD	SD	MIN	MAX
Biotix – 1	4	0.434	0.016	0.412	0.446
Biotix – 1:1	4	0.443	0.021	0.422	0.462
Biotix – 1:4	4	0.450	0.017	0.428	0.464
Biotix – 1:8	4	0.503	0.015	0.487	0.516
Axygen – 1	4	0.418	0.025	0.396	0.449
Axygen – 1:1	4	0.417	0.014	0.398	0.430
Axygen – 1:4	4	0.443	0.013	0.417	0.457
Axygen – 1:5	4	0.468	0.025	0.454	0.493
MBP ART – 1	4	0.418	0.030	0.385	0.449
MBP ART – 1:1	4	0.447	0.034	0.428	0.460
MBP ART – 1:4	4	0.443	0.014	0.438	0.452
MBP ART – 1:8	4	0.489	0.013	0.453	0.538
Positive Control	8	0.517	0.022	0.475	0.542
Negative Control	2	0.066	N/A	N/A	N/A

**Figure 8: Dose Response for Cell Toxicity**



**CONCLUSION**

Comprehensive testing by independent research laboratory, MRIGlobal, demonstrated the ability of Delta Filter technology in Biotix pipette tips to provide excellent aerosol blocking efficiency across a broad range of highly contaminating substrates including PCR amplicons, bacteria, viruses, and nanoparticles. Delta Filter provides the security expected of leading filter tips on the market, with the added security of instant visual identification of pipetting errors with the color change filter technology. Biological testing highlighted the chemically inert Delta Filter is suitable with even the most sensitive assays.

For more information about Delta Filter and Biotix pipette tips, visit us at our website or contact us at the address below.

**References**

- (1) Dieffenbach, C., and Dveksler, G.S., "Setting Up a PCR Laboratory," *PCR Primer: A Laboratory Manual*, Cold Spring Harbor, New York, 2003, pp.12.
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- (3) Bagg, A., "Molecular Pathology Methods – Equipment," *Molecular Pathology in Clinical Practice*, Springer LLC., New York, 2007, pp. 560.
- (4) Coleman, W.B., and Tsongalis, G., "Framework for Quality Assurance in Molecular Diagnostics," *Molecular Diagnostics: For the Clinical Laboratorian*, 2<sup>nd</sup> edition, Humana Press, New Jersey, 2010, pp.231.