APPLICATION NOTE

Maintaining Sample Integrity with Aerosol-blocking Tips

Liquid Handling Consumables, Thermo Fisher Scientific

Abstract

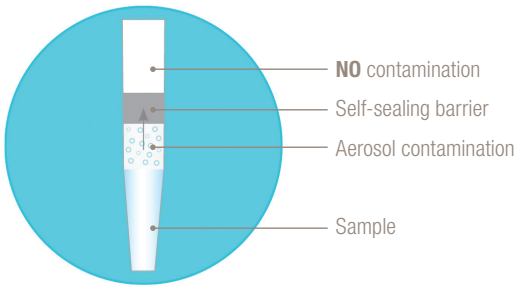
Thermo Scientific™ ART™ self-sealing barrier tips were tested by an independent laboratory to demonstrate their ability to preserve sample integrity during use in PCR. First, the ability of the proprietary ART barrier to prevent contamination was tested and compared to other filter tips. Second, the ability to recover liquid which was overdrawn into the ART barrier was determined. Third, the ART barrier was tested to show that it does not compromise or inhibit PCR if accidentally contacted by samples used in PCR.

Introduction

Preserving the integrity of PCR conditions and samples is essential. For this reason, Thermo Fisher Scientific developed ART self-sealing barrier tips to prevent contamination and provide reproducible sample delivery.

It is important to select an aerosol-blocking tip based on its ability to prevent aerosols and liquids from contaminating the pipettor or subsequent samples. A total of three brands of tips were used in this test, but only ART self-sealing barrier tips were able to prevent contamination.

In the event the sample contacts the filter of a tip, it may be necessary to retrieve the sample. To prove the ability of the ART barrier tip to minimize sample holdback, retention was quantified using water.



Because of the sensitivity of PCR, a variety of factors can compromise the integrity of the reaction. This illustrates the importance of using aerosol-blocking tips which do not inhibit PCR or otherwise introduce contaminants into precious samples. To demonstrate that ART self-sealing barrier tips do not inhibit PCR, an independent laboratory evaluated ART barrier tips using protocols that duplicated actual laboratory conditions.

Objective

The independent laboratory tests demonstrate that only ART self-sealing barrier tips effectively prevent aerosol and liquid contamination, minimize sample retention, and that the proprietary ART barrier does not inhibit PCR when used as intended.

Materials and Methods

Experiment #1 - Contamination Study

To ensure that all tips used were initially contaminant-free, all brands were tested for the presence of human DNA.

Sample to pipettor contamination

Using a Gilson™ Pipetman™ P-200, prior to amplification 200 µl of PCR mix containing 20 ng of total human genomic DNA from blood was drawn up and through (when possible) three different brands of aerosol-blocking tips. The liquid was then collected from the pipette side of the filters with a DNA-free tip.

Pipettor to sample contamination

Two microliters of DNA (10 ng/µl) was dispensed on top of the filters of three different brands of 20 µl aerosol-blocking tips. Then by setting a Pipetman P-200 to 200 µL, approximately 20 µL of dH2O was drawn through the filters (when possible) and allowed to contact the DNA sample.

In both tests, the resulting liquid was tested by PCR for the presence of DNA with primers specific for human Alu gene. Thermal cycling consisted of 40 cycles of 94° C (1 min), 40° C (1 min), and 72° C (1 min). The resulting PCR products were analyzed on a 1.2% agarose gel in 1X TAE.

Experiment #2 - Sample Retention Study

Ten ART 20 µl tips were weighed individually to determine their initial weight. Forty microliters of water was then overdrawn to intentionally contact the barrier, then quickly dispensed (quick dispensing allows sample to be retrieved prior to the barrier sealing). The tips were then weighed to determine the amount of water retained, if any.

Experiment #3 - PCR Inhibition Study

Using a Pipetman P-200 set at 60 µl, dH2O was drawn into a 20 µl ART barrier tip. After contacting the ART barrier, the extract was retrieved and 20 µl was used in a 30 µl PCR reaction (2.5 U) Taq DNA Polymerase, primers for the human Alu gene, 30 pg human genomic DNA template, PCR buffer (10 mM MgCl2). A total of 10 ART tips were tested using this process. Negative and positive controls were also performed.

Thermal cycling consisted of 40 cycles of 94° C (1 min), 40° C (1 min), and 72° C (1min). The resulting PCR products were analyzed on a 1.2% agarose gel in 1X TAE.

Results

Experiment #1

Because of the self-sealing properties of the ART barrier, no DNA was able to pass through in either direction. This resulted in an absence of DNA product in the final analysis (Figure 1). However, the presence of DNA contamination with Brands B and C indicates that with the remaining two filter tips, DNA passed through the filter in both directions.

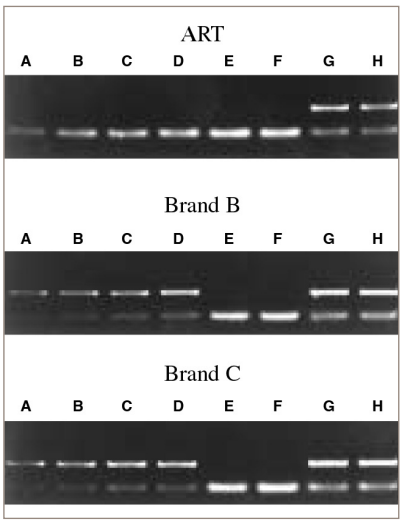


Figure 1

All Gel Photos - Lanes A,B: Sample to pipettor contamination - DNA pulled through barrier; Lanes C,D: Pipettor to sample contamination - DNA expelled down through barrier; Lanes E,F: Negative controls; Lanes G,H: Positive controls

Experiment #2

After weighing each ART barrier tip individually, it was found that an average of 96.9% and as much as 98.5% of the sample was recovered after over-pipetting (Table 1).

ART Tips	% Of Sample Recovered
1	98.0
2	97.5
3	98.5
4	96.3
5	96.5
6	97.0
7	95.5
8	95.0
9	97.3
10	97.0
AVG.	**96.9%**

Table 1

thermo scientific

Experiment #3

As seen in Figure 2, the material in the proprietary ART barrier does not inhibit PCR. The resulting product utilizing dH2O, which came in contact with the ART barrier, shows equal band intensity when compared to the positive control.

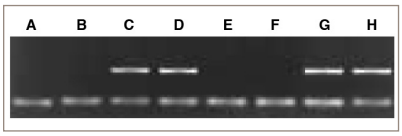


Figure 2
SAMPLES- Lanes A,B: Water which contacted the ART barrier and PCR reaction mixture without DNA; Lanes C,D: Water which contacted the ART barrier and DNA in PCR reaction mixture.

CONTROLS - Lanes E,F: Extract without DNA in PCR reaction mixture; Lanes G,H: Extract with DNA in the PCR reaction mixture.

Discussion

Maintaining sample integrity is essential to obtaining accurate experimental results, especially in highly sensitive reactions like PCR. In determining which aerosol-blocking tip to use in the laboratory, it is important to evaluate its ability to prevent carryover contamination.

In Experiment #1, it was demonstrated that aerosol-blocking tips utilizing a porous plastic filter allow the passage of DNA in solution in both directions. Only ART barrier tips have the self-sealing capability necessary to prevent such contamination.

Some experiments require expensive reagents and scarce or limited-availability samples. In the unlikely event that an overpipetted sample contacts the ART barrier, it can be easily retrieved. In Experiment #2, the data shows that as much as 98.5% of the overdrawn sample was recovered.

Numerous factors can result in the inhibition of PCR. Among these are excesses of certain reagents or exposure to certain environmental conditions. In Experiment #3, it was demonstrated that the self-sealing barrier in ART pipette tips neither compromises nor inhibits PCR in standard conditions.

Finally, wherever there is a risk of filter contact with samples, the security of knowing when samples have touched the filter is the most important benefit of any filter tip system. ART self-sealing barrier tips are the only tips that seal completely to let the user know when a sample has touched the filter. When the barrier seals it automatically warns the user of potential sample contamination.

Australia +61 39757 4300
Austria +43 1 801 40 0
Belgium +32 53 73 42 41
China +86 21 6865 4588 or +86 10 8419 3588
France +33 2 2803 2180
Germany National Toll-Free 0800 1 536 376
Germany International +49 6184 90 6000
India Toll-Free 1800 22 8374
India +91 22 6716 2200
Italy +39 02 95059 554
Japan +81 45 453 9220
Netherlands +31 76 579 55 55
New Zealand +64 9 980 6700
Nordic/Baltic/CIS Countries +358 9 329 10200
Russia +7 812 703 42 15
Spain/Portugal +34 93 223 09 18
Switzerland +41 44 454 12 22
UK/Ireland +44 870 609 9203
USA/Canada +1 866 984 3766
Other Asian Countries +852 2885 4613
Countries Not Listed +49 6184 90 6000

Find out more at **thermofisher.com/pipettes**

ThermoFisher SCIENTIFIC

ANHPTARTIntegrity0516