

Maintaining Sample Integrity with Aerosol-Blocking Tips

ABSTRACT

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 patented 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.

INTRODUCTION

Preserving the integrity of your PCR conditions and samples is essential. For this reason, Molecular BioProducts, Inc., 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 perform as indicated. Specifically, an aerosol-blocking tip should be selected for 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 your 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 tips using protocols that duplicated actual laboratory conditions.

OBJECTIVE

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

MATERIALS & METHODS

Experiment #1

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 Pipetman[®] P-200, 200 μ l of PCR mix (prior to amplification) containing 20 ng of total human genomic DNA from blood was drawn up and through (when possible) three different brands of aerosolblocking tips. The liquid was then collected from the opposite side of the filters with a DNA-free tip.

Pipettor to sample contamination

Two microliters of DNA (10 ng/ μ l) was dispensed on top of the filters of three different brands of 20 μ l aerosol-blocking tips. Approximately 20 μ l of dH₂O was then drawn through the filters (when possible) and allowed to contact the DNA sample by setting a Pipetman P-200 to 200 μ l. This liquid was then dispensed back through the filter.

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 $^{\circ}$ C (1 min), 40 $^{\circ}$ C (1 min), and 72 $^{\circ}$ C (1 min). The resulting PCR products were analyzed on a 1.2% agarose gel in 1X TAE.

Experiment #2

Ten ART 20P 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's sealing). The tips were then weighed to determine the amount of water retained, if any.

Experiment #3

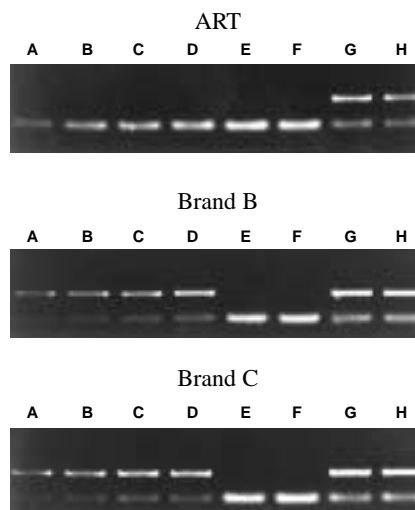
Using a Pipetman P-200 set at 60 μ l, dH₂O was drawn into a 20 μ l ART tip. After contacting the ART barrier, the extract was retrieved and 20 μ l was used in a 30 μ l PCR reaction (2.5 U Taq DNA Polymerase, primers for the human Alu gene, 30 pg human genomic DNA template, PCR buffer (10 mM MgCl₂)). 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 $^{\circ}$ C (1 min), 40 $^{\circ}$ C (1 min), and 72 $^{\circ}$ 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 ability 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 (see below). However, the presence of DNA contamination with Brands B and C indicates that with the remaining two filter tips, DNA passed through in both directions.



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.

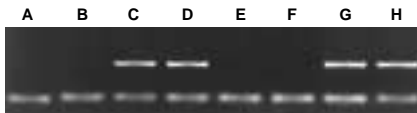
Experiment #2

After weighing each ART 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 (see below).

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

Experiment #3

As seen below, the material in the patented 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.



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

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. Only ART tips have the self-sealing capability necessary to prevent such contamination.

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 pipet tips neither compromise nor inhibit PCR in standard conditions.

Finally, wherever there is a risk of filter contaminated 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 let the user know when a sample has been touched by the filter. When the barrier seals it automatically warns the user of potential sample contamination.

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