

Abstract

Low retention pipet tips and standard pipet tips were compared by an independent laboratory. The assay compared the recovery of an oligonucleotide to determine the retrievability of pipetted samples. The binding of 32P labeled oligonucleotides to pipet tips was measured. The new low retention tips supported Molecular BioProducts' claims by increasing sample recovery and performing more precisely than standard pipet tips.

Introduction

Pipet tip sample retention is a major factor in pipeting accuracy, creating the need for a low retention pipet tip. The variation of this retention is also a major factor that leads to inconsistent data. With reagents becoming increasingly more expensive, it may be more cost effective to utilize the low retention pipet tips depending on your specific testing. Furthermore, decreased retention will increase analytical accuracy of many methods, such as Polymerase Chain Reaction. The purpose of this study is to examine the effectiveness of low retention pipet tips compared to standard pipet tips.

Objective

The objective of this experiment was to verify that the new low retention tip will increase the efficiency of the users' assays by reducing sample retention inside the tip. We are also testing the low retention tips to show that they are more consistent in their binding characteristics and result in more precise aspirations.

Materials and Methods

Experiment 1:

An oligonucleotide (HAV3: TGTGCCGAATCTATTGGGGTCCAGC) was labeled with 32P at the 5' – hydroxyl-terminal by T4 polynucleotide kinase. The unincorporated 32P – ATP was removed from the labeled HAV using spin column chromatography. A 1:100 dilution

was made for the 32P – labeled HAV oligonucleotides and used in this assay. Ten low retention pipet tips and ten standard pipet tips were tested in this assay. In addition, in order to determine the counts per minute (CPM)/ul of stock HAV, 1 ul of 32P- labeled HAV was transferred to a scintillation vial containing scintillation fluid for the measurement of total CPM.

The tips were pipetted with 32P labeled HAV oligonucleotides, and the sample was discarded immediately. The tips were then washed 5 times with 100 ul of Tris-EDTA buffer. The tips were put into scintillation vials containing scintillation fluid for the measurement of CPM.

Results

Experiment 1:

As shown in Table 1-1 below, oligonucleotide HAV shows a mean CPM retention of 1451 CPM in the low retention tips. In the standard tips, 3611 CPM was observed.

The results of HAV probe's mean CPM using standard tips shows a 149% greater retention of the oligonucleotide HAV when compared with the mean CPM using the low retention tips. This means that the standard tip results in 2.5 times the retention of that seen with the low retention tips. This 1:100 solution left 11% of the total HAV 32P labeled oligonucleotides in the standard pipet tips as compared to 4.4% in low retention tips.

Table 1-1: Summary of HAV probe results
(Mean CPM is counts associated with tip after washing)

	Diluted HAV probe	
	Low Retention	Standard
Mean CPM	1451.3	3611.0
SD CPM	684.8	2336.4
% Total added	4.4	11.0

*Stock HAV probe is @ 654489 CPM/ul

Perhaps more importantly, the low retention tips were more precise in the amount of oligonucleotide retention. The standard deviation in the amount of oligonucleotide held in the tips was just 684.8 CPM in the low retention tips versus 2336.4 CPM in the standard tips.

Discussion

In determining which pipet tip to use in your laboratory, it is important to evaluate the level of precision and accuracy you need. The results of this experiment show that the low retention tips are more accurate in dispensing the sample pipetted. This should save on reagent costs and improve efficiency in your experimentation. The results also demonstrated that the low retention tips are more consistent in the amount of oligonucleotides that adhere to it. This will increase the precision of your pipetted sample, which should yield more consistent results in your experimentation.

In the future this study could be conducted with a larger sample size and over various substances, such as proteins. It should also be determined if the advantages in accuracy and precision of the low retention tips will improve the outcome of an experiment. Based on the positive results seen in the low retention tips, a low retention tube may also be beneficial for laboratories.

In conclusion, the low retention tips showed a distinct advantage in increasing accuracy by dispensing more of the aspirated oligonucleotides. The tips were also more consistent in the amount of oligonucleotides retained.