Purification of Radiolabeled Oligonucleotides Using ZipTipC_{18} Pipette Tips

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Introduction

ZipTip is a 10 µL (P-10) pipette tip with a bed of chromatography media fixed at its end such that there is no dead volume. It is intended for purifying and concentrating femtomoles to picomoles of protein, peptide or oligonucleotide samples prior to analysis, providing better data quality. The sample is aspirated and dispensed through ZipTip to bind, wash, and elute. Recovered samples are contaminant-free and eluted in 0.5-4 µL.

This protocol provides a guideline for using ZipTip$_{C18}$ to purify radiolabeled oligonucleotides away from free radiolabeled nucleotide and buffer solution, which may be incompatible with further oligonucleotide manipulations such as probing, bandshift assay and primer extension.

Materials

- ZipTipC18 pipette tips
- P-10 pipettor, multi-channel P-10 pipettor (Biohit ProlineÒ pipettor recommended) or compatible automated liquid handling work station
- Wetting solution: HPLC-grade acetonitrile
- Equilibration solution: 1X labeling buffer (70mM Tris HCl (pH 7.6), 10mM MgCl2, 5mM DTT)
- Wash solution: ultrapure water/ 3% acetonitrile
- Elution buffer: 30% ACN/ ultrapure water

NOTE: Since the resin bed provides a slight back-pressure, the pipette should not be used as an accurate volumetric dispenser. To achieve optimal sample uptake and delivery, set pipettor to 10 µL and attach tip securely. Depress plunger to dead stop and slowly release or dispense plunger throughout operation.

Procedure

Prepare the Sample:
Dilute the radiolabeled sample to 20 µL with the **equilibration solution.**

Equilibrate the ZipTip for Sample Binding:
1. Prewet the tip by depressing pipettor plunger to a dead stop using the maximum volume setting of 10 µL. Aspirate **wetting solution** into tip. Dispense to waste. Repeat twice more.

2. Equilibrate the tip for binding by washing with 20 µL of **equilibration solution** 3 times.

Bind and Wash the Oligonucleotides:
Follow these steps after equilibration:
1. Bind oligonucleotides to ZipTip$_{C18}$, by fully depressing the pipettor plunger to a dead stop. Aspirate and dispense sample 5 to 10 cycles.

2. Wash tip 3 times with 10 µL of fresh **wash solution #3**, dispensing to waste after each aspirate and dispense cycle.

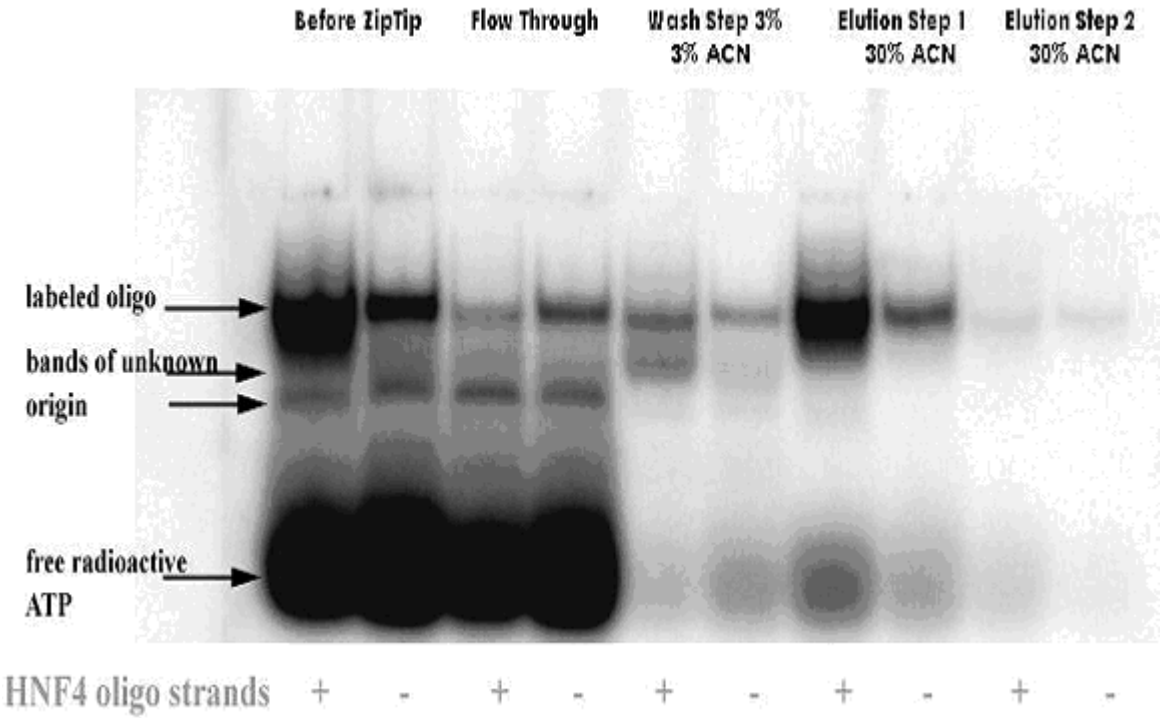
Elute the Oligonucleotides:
For ZipTip$_{C18}$, dispense 1 to 10µL of **elution solution** into a clean vial using a standard pipette tip. Carefully, aspirate and dispense eluant through ZipTip at least 3 times without introducing air. Sample recovery can be improved (at the expense of concentration) by eluting in the higher volume of 10 µL.

CAUTION: Acetonitrile is volatile and evaporation can occur rapidly. If this occurs, add more eluant to recover sample.

To prepare oligonucleotides for use as radioactive probes or for bandshift after ds annealing.
1. Recover the oligonucleotide by evaporating the 10uL eluant to dryness in a centrifugal evaporator (Savant SpeedVac or its equivalent).

Redissolve the radiolabeled oligonucleotide in a small volume (10uL) of TE (pH 7.6).

Results



20ml of g-P32 labeled ATP was cleaned up using by binding the sample to a ZipTip$_{c18}$ washing with 3% Acetonitrile to remove unincorporated label, and Eluting with 30% Acetonitrile twice. Samples from each step were run out on a polyacrylamide gel to evaluate the removal of the unincorporated label.

For more information on using ZipTip$_{C18}$ visit Frequently Asked Questions (FAQ's) at www.millipore.com/ziptip. Go to the bottom of the page and choose FAQ's from the "Technical Library" pull-down menu.