Trifluoroacetic Acid (TFA) Salt Exchange Removal Protocol

About

When peptides are freeze dried, they dry as peptide trifluoroacetate salts. This trifluoroacetic acid (TFA) remains in the sample from its use as an ion-pairing agent during purification, as well as from the cleavage from resin. For certain applications it is valuable to remove the TFA. This can not only change the structure of the peptide in solution, but it can also make the peptide toxic to cells and significantly adjust the pH when dissolved. The following protocol details the exchange of the TFA salts with chloride ions from hydrochloric acid (HCl). If HCl cannot be used for the peptide's applications, then other salts may be used; however, the efficiency of the exchange is not guaranteed.

Glassware and Equipment

- 50-mL Conical Tubes
- High Performance Liquid Chromatography (HPLC) Fractions with Purified Peptide
- 1 x Vortex Mixer
- 1 x Adjustable 1000-µL Micropipette

Materials

The materials needed for this protocol are provided below. The Fisher Scientific catalog numbers are provided in parentheses.

- 1 M Hydrochloric Acid Solution (A144-500)

Safety Measures

When performing this protocol, users must wear safety glasses, laboratory gloves, pants, closed-toe shoes, and a fire-retardant laboratory coat. These chemicals have the following hazard identifications:

Hydrochloric Acid (HCl): 

Procedures

1. Begin by obtaining the purified peptide in high-performance liquid chromatography (HPLC) fractions.
    a. If the peptide is not dissolved in aqueous solution, the purified peptide should be dissolved in Milli-Q water.
    b. Peptide concentration is not particularly important; however, if dissolving peptide, a 0.1 mM concentration should be adequate.
2. Determine the volume of 1 M HCl that should be added to each conical tube to make a ~25 mM HCl solution.
3. Using a micropipette, add the required volume of 1 M HCl to each of the conical tubes.
4. Vortex the conical tube(s) to ensure adequate mixing.
5. The TFA salt exchange is now completed. The solution can then be flash frozen and lyophilized.
    a. If the lyophilized peptide is very crystalline, then it is worth redissolving in water, which will dilute the HCl. Re-lyophilizing should result in the ideal fluffy lyophilized powder.