C$_{18}$ ZipTip Peptide Clean-up Protocol for LC-MS/MS

(NOTE: This will NOT remove detergents)

For most applications, use 100 µL ZipTips

**Solution Prep (daily)**
- Solution A: 100% water + 0.1% TFA
- Solution B: 50% water + 50% acetonitrile (ACN) + 0.1% TFA
- Solution C: 25% water + 75% ACN + 0.1% FA

**Wet and Equilibrate Tip**
- Aspirate and Dispense tip 2 times with 100 µL of Solution B
- Aspirate and Dispense 3 times with 100 µL of Solution A

**Sample Binding**
- Bind up to 200 µL of sample by cycling (aspirate-dispense-aspirate-dispense) 5-10 times (IMPORTANT: Be sure no organic solvent is present in sample)
- Aspirate and Dispense 2 times with 100 µL of Solution A to wash sample

**Eluting**
- Elute with 100 µL of Solution C
- Lyophilize in SpeedVac
- Submit dry sample to MS facility

**Note:**
- Before Zip tip processing, stop the trypsin digestion by adding TFA to a final concentration of 0.1%
- In order to retrieve most of the peptides in your sample, repeat these steps with new ZipTips (3-5 times) and combine eluted peptides.