## Overview and Considerations:

We typically use three methods for protein precipitation, each have advantages and disadvantages. 1) Acetone is reasonably fast and will precipitate free GlcNAc; 2) Methanol is slow, and does not precipitate GlcNAc; 2) Methanol Chloroform is reasonable fast, but does not work well in the presence of free peptide. Your choice will depend on the volume of your sample, the time available, and the presence of free GlcNAc/peptide.

**IMPORTANT:** Organic solvents are not compatible with all of the centrifuge tubes in the laboratory. Check with the manufacturer before using for the first time.

**IMPROTANT:** This technique works best in 15ml conical tubes.

## Reagents:

1. Ice Cold Acetone.
2. Microcentrifuge, 4oC
3. Speed-vac.

## Protocol:

1. Add 10 volumes of ice cold acetone to your samples (to 100ul of sample add 300ul of acetone);
2. Vortex to mix;
3. Incubate 2h-overnight at -20oC;
4. Pellet protein at full-speed in a microfuge at 4oC for 30min (reduce to 3000xg for 15ml tubes);
5. Decant supernatant;
6. Dry samples briefly in a speedvac, ~5-10min.
7. Resuspend samples in sample buffer. I usually place samples on the vortex for 5-10min.

*Note: if your gel samples turn yellow when you add loading buffer, you have residual acetone in your samples and they need to be dried further.*