**Guanine Nucleotide Exchange Factor (GEF) Assay SOP**

NOTE: This assay is very sensitive to salt, pH, and temperature. Make sure all proteins are in the same buffer before you run the assay.

NOTE: The buffer that I found worked well is 10mM HEPES pH 7.0, 50mM NaCl, 2mM MgCl2, and 1mM DTT. I turned on the temperature control on the fluorimeter, but the plate reader should be relatively insensitive to temperature change.

NOTE: This reaction is based on Mant-GTP fluorescence. When Mant-GTP is loaded into the GTPase, you will see an increase in fluorescence.

NOTE: You will need to optimize protein concentrations for your assay. I found that 0.4uM Tiam1 GEF worked well for me, but I had to use 10uM Vav2 GEF to see similar activity. You will need to do a titration to see. You may also need to vary the GTPase concentration and the Mant-GTP concentration.

NOTE: GEF activity is dependent on the DH domain, but almost all GEFs contain a tandem DH-PH domain. The PH domain helps increase the activity of the GEF protein, removing it will decrease the activity of the protein.

1. Start up the fluorimeter or the plate reader. Set the excitation to 350nm and the emission to 440nm, read every 2s for ~10 minutes (the reaction is incredibly quick and will be over before that)
2. Make Mix I and add it to the cuvette and start reading. The components are:
   1. GEF Buffer to fill the volume to 150uL
   2. GTPase at the appropriate concentration (I used 10uM protein)
   3. Mant-GTP to the final concentration (I used 2uM)
3. While Mix I is reading, make Mix II. The components are:
   1. GEF Buffer to fill the volume to 150uL
   2. GEF protein to the appropriate concentration (I used 0.4uM for Tiam1 and 10uM for Vav2).
   3. Mant-GTP to the same concentration as in Mix I
      1. NOTE: c is critically important. If you do not add Mant-GTP to both mixes the fluorescence will drop from a reduction in concentration, not anything to do with your assay.
4. Read for ~150seconds and then add Mix II to Mix I, pipetting very carefully to avoid bubbles
   1. If you are using a cuvette, you will have to use a gel loading tip to mix
5. Almost immediately, you should see a steep increase in the fluorescence.
6. Keep reading until the reaction has plateaued