

### **Kynurenine in supernatant**

Kynurenine is soluble in 0.5M HCl and could be stored in aliquots of 1mL at -20 degrees. The stock solution is 50mM. (Clear dark yellow to yellow-orange)

- Make a standard beginning with 5000µM L-Kynurenine (Stock is 50mM)
- 60µl sample/Standard + 30µl 30% trichloroacetic acid (TCA)
- 30min 50C (cover the plates before incubating)
- 10min Spin, 3000 x g
- Take sup (70-80 µl) and add 1:1 to freshly prepared Ehrlich Reagent (2% p-dimethylaminobenzaldehyde in glacial acetic acid , ie 0.2g powder+10ml acid)
- 12-30min
- measure absorbance at 492nm

### **Another Protocol:**

- Make a standard similar to that of nitric oxide. (0-100µM)
- 100µl sample/Standard + 50µl 30% trichloroacetic acid (TCA)
- vortex and centrifuge at 8000 x g for 5 min
- take 75µl of the supernatant and mix with equal volume of Ehrlich's reagent
- Ehrlich's reagent (100mg of p-dimethylaminobenzaldehyde in 5ml glacial acetic acid)
- Place in microtiter plate and read at 492nm after 15 min.

### **Another protocol:**

- 1) 160 µl of the cell supernatant/standard were removed from each well and transferred to a corresponding well of a 96-well round-bottom culture plate.
2. After addition of 10 µl 30% (v/v) trichloroacetic acid to each well, the plates are incubated for 30 min at 50° C to hydrolyze N-formylkynurenine to kynurenine.
3. Centrifuge at 3000 x g for 10 min (600 x g for 10 min)
4. Transfer 100 µl of supernatant to wells of a 96-well flat-bottom plate
4. Mixed with 100 µl of freshly prepared Ehrlich's reagent. (1.2% w/v 4-dimethylamino)benzaldehyde in glacial acetic acid)
6. Incubate for 10 min at r.t.
5. Read absorbance with a microplate reader at 492 nm.
6. Use a blank that containing culture media only.