Detection of Arginase-1 expression through Urea assay


Need to purchase

- Quantichrom urea assay kit (Bioassay Systems, cat. no. DIUR-500)

Solutions to prepare (recipes):

**Arginase activation solution**

- Mix 50 μl of 1 M MnCl₂ (Sigma-Aldrich) and 250 μl of 1 M Tris·Cl, pH 7.5 in 4.7 ml water to make up a 10 mM MnCl₂/50 mM Tris·Cl, pH 7.5 solution.
- Store indefinitely at room temperature.

**Arginine substrate solution**

- Add 871 mg of L-arginine to 8.5 ml water. Adjust pH with ~1 ml of 1 M HCl to pH 9.7 to make up a 0.5 M L-arginine, pH 9.7 solution.
- Adjust volume with water to 10 ml. Store indefinitely at room temperature.

**Lysis buffer**

- 0.001% Triton X-100 (Sigma-Aldrich), 1× protease inhibitor cocktail
- Mix 5 μl of Triton X-100 in 4.8 ml distilled water and add 200 μl of 25× protease inhibitor cocktail solution. Prepare fresh.
  ***Lysis buffers we used for WB, should work fine. As long as it is fresh or had been stored at -20°C

**HCl (1 M)**

- 913.8 ml H₂O + 86.2 ml concentrated HCl

**MnCl₂ (1 M)**

- 9.89g in 50mL of ddH2O

**Tris·Cl (1 M)**

- Dissolve 121 g Tris base in 800 ml H₂O
- Adjust to desired pH with concentrated HCl
- Adjust volume to 1 liter with H₂O
- Filter sterilize if necessary
- Store up to 6 months at 4°C or room temperature
Approximately 70 ml HCl is needed to achieve a pH 7.4 solution, and ~42 ml for a solution that is pH 8.0.

**IMPORTANT NOTE:** The pH of Tris buffers changes significantly with temperature, decreasing approximately 0.028 pH units per 1°C. Tris-buffered solutions should be adjusted to the desired pH at the temperature at which they will be used. Because the pKₐ of Tris is 8.08, Tris should not be used as a buffer below pH ~7.2 or above pH ~9.0.

**Urea standard solution**
- Dissolve 500 mg urea (Sigma-Aldrich) into 5 ml of water to make up a 100 mg/ml urea solution.
- Store for 1 year at room temperature.
- Adjust the highest, starting concentration of the urea standard from 100 to 5 mg/ml to match the experimental samples.

(*I don’t use the urea standard provided in the kit and use the recipe above to make my standard. In doing so, I’m able to extend the range of my curve. I create my curve by using serial dilutions of my standard – 1:4 - 8 times.)*

**Blank solution**
- ¼ Arginase activation solution + ¼ Lysis buffer + ½ Arginase substrate solution
- Prepare at whatever volume, as long as the ratio is constant.
**PROCEDURE**

1. Sample preparation:
   - Harvest macrophages, ~48 hours after stimulation.
   - Wash cells with PBS → Spin → remove PBS → new tube
   - Lyse cells with lysis buffer (the volume used will vary depending on how many cells you obtained. I.e. ~1x10^6 - 2x10^6, I typically add 100μL.
   - Lyse cells with harsh pipetting and place tubes on rock plate for 15’ at room temperature
     ***If you do not wish to do this, you can prepare protein samples as you would total protein for Western blot analysis.

2. Arginase activation
   - 50μL of Arginase activating solution + 50μL of sample (could be more or less, as long as the ratio is consistent 1:1)
   - Mix and *incubate* for 10’ at 55C

3. Urea production
   - Take 50μL of activated samples and add 50μL of *arginase substrate solution*
   - Incubate at 37C for ~2 hours
   - If the concentration of urea produced is predicted to be low, incubate for longer (~up to 24 hours). Don’t be alarmed if you see brown precipitate forming ~6hours into the incubation. Its jus normal precipitate from the MnCl2).

4. Assay
   - Create Urea standard curve (see note on “Urea standard solution” – recipe)
   - Mix 1:1 ratio of solution A and B provided in the kit (Kit says use 200μL of Solution A+B mix, but I found 100μL of the mix was sufficient)
   - Take 5μL of serially diluted urea standards and add 100 or 200μL of solution A+B mix. Place on 96 well plate.
   - Take 5μL of samples and add 100 or 200μL of solution A+B mix. Place on 96 well plate.
   - Prepare a *blank control* and add 100 or 200μL of solution A+B mix. Place on 96 well plate.
   - Incubate in the dark (i.e. cover with tinfoil) for anywhere between ~2-20 mins . If urea is present, it will turn a very bright orange. The more concentrated the faster the reaction will be.
   - Check absorbance at 520nm!