**8M Urea Sample buffer preparation protocol:**

**Materials:**

* Urea - bromophenol blue
* Thiourea - Tris
* Glycerol - Resin ([Rexyn 300](https://andwinsci.com/product/uncategorized-items/rexyn-300hoh-cert-16-50m-500g-1227746))
* SDS - Leupeptin and PMSF
* DTT - 0.22 µm [Analytical Filter Funnel](https://www.thomassci.com/Laboratory-Supplies/Filtering-Funnels/_/ANALYTICAL-TEST-FILTER-FUNNELS)

**Methods:**

**\***[**Use amounts calculated on spreadsheet**](#Urea_Buffer) **and follow steps below\***

1. Combine Urea and Thiourea in a clean beaker and dissolve in 10% of deionized water
   * Do not add too much water, the urea / thiourea takes up over half of the final volume
2. Stir gently in a hot plate until the solution is dissolved at room temperature.
   * If needed, heat the solution until dissolved but DO NOT heat above 40°C (this will help avoid cyanate formation from heating Urea)
   * Be patient, this takes time for the Urea and Thiourea to dissolve completely
3. Add 10% (w/v) of a mixed bed resin (Rexyn 300) to the solution and stir at room temperature for 15 minutes
   * This will deionize the urea buffer, remove cyanate and any other ionic constituents
   * If you have a conductivity meter, the conductivity should be less than 5 µmhos
4. Filter the mixture through the 0.22 µm Analytical Filter Funnel (Thomas Scientific - 4618U76) to remove the resins.
5. Add the Tris-base and SDS
6. Adjust pH to 7.5 using HCl.
7. Add solid DTT and stir until dissolved.
8. Continue to adjust the pH down to 6.8 using HCL.
9. Add Bromophenol blue and stir until dissolved.
10. Bring up to the final volume by adding deionized water
11. Aliquot into 4 mL in cryogenic vials microfuge vials, and stored at -20°C for future use
    * Each tube should be labelled “8 M Urea Buffer / Initials / Date”
12. For the [50% Glycerol solution](#Glycerol), add Glycerol (50% v/v) and protease inhibitors (E64, Leupeptin, and PMSF) into a clean beaker
13. Bring up to the final volume by adding deionized water
    * This will also help dissolve the protease inhibitors in solution
14. Once dissolved, aliquoted into 4 mL in cryogenic vials microfuge vials, and stored at -20°C for future use
    * Each tube should be labelled “50% Glycerol + protease inhibitors / Initials / Date”