Overview

These document explains the materials necessary steps for a urea thiourea sample buffer.

Additional resources

Need more help?

Check the resources, and then see Ken

Main content

**Materials**

* 8M Urea: Sigma, U6504-500G
* 2M Thiourea: Sigma, 88810-100G
* 0.05M Tris: Fisher, BP152-1
* 75mM DTT: Fluka, 43819-5G
* 3% SDS: Biorad,161-0301
* Rexyn 300: Biorad, R276-500
* Filter paper
* 0.22 µm filter
* 150 mL glass beaker

**Methods**

Sample Buffer Preparation (for 100 mL volume)

1. Use gloves when making this solution
2. Weigh 48 grams of urea and 15.2 g of thiourea into a clean 150 ml glass beaker. Add 40ml of water and stir gently on a hot plate until the solution is at room temperature and all the solids are dissolved. Careful not to heat above 40 ºC
3. Add 10 grams of mixed bed resin, stir mixture at RT for 15 minutes
4. Filter mixture through a filter paper into a 100 ml graduated cylinder. Carefully rinse the resin with two 5 ml aliquots of DI water. Transfer the filtered solution back to a clean beaker
5. Weigh 0.605g of Tris base and 3g of SDS, add to the solution and stir until its dissolved. Adjust pH down to 7.5 using 12 M HCl. (~ 300-400 ul)
6. Add 1.155g of solid DTT and stir it until its dissolved
7. Adjust pH down to 6.8 using 2M HCl. Use 10-20 ul. If you mistakenly go past 6.8, correct the pH up using 2 M Tris base.
8. Transfer solution to graduated cylinder. Add DI water to top it off to 100ml. Mix. Filter through a 0.22 µm filter to remove any fine particulate matter.
9. Make 2 ml aliquots and freeze at -80 ºC, thaw amount as needed just before use.