# Protocol: Preparation of E. coli S-100 Extract for Luciferase Assay (Jill Thompson)

**IMPORTANT:** Everything should be done at 4 ºC! Use ice-cold Buffers, Rotors, etc. Use RNase free lab wares and reagents.

**Materials**

**Wash Buffer**

| Stock | Final Conc | Volume (1L) |
|---|---|---|
| 1M HEPES-KOH, pH 7.5 | 10 mM | 10 ml |
| 1M MgCl2 | 10 mM | 10 ml |
| 2.5M NH4Cl | 1000 mM | 400 ml |
| Mercaptoethanol | 5 mM | 350 ul |
| Water | | 580 ml |

**S-30 Buffer**

| Stock | Final Conc | Volume (50 ml) |
|---|---|---|
| 1M HEPES-KOH, pH 7.5 | 10 mM | 500 ul |
| 1M MgCl2 | 10 mM | 500 ul |
| 2.5M NH4Cl | 50 mM | 1000 ul |
| Mercaptoethanol | 5 mM | 18 ul |
| Water | | 48 ml |

**S-30 Buffer + 10% glycerol**

| Stock | Final Conc | Volume (1L) |
|---|---|---|
| 1M HEPES-KOH, pH 7.5 | 10 mM | 10 ml |
| 1M MgCl2 | 10 mM | 10 ml |
| 2.5M NH4Cl | 50 mM | 20 ml |
| 100% Glycerol | 10% | 100 ml |
| Mercaptoethanol | 5 mM | 350 ul |
| Water | | 860 ml |

**100 mM Glycine-KOH, pH 9.2:**
75 mg of glycine (F.W = 75) in 10 ml water. First pH with KOH to 9.2 before final volume is made to 10 ml.

**25 mM EGTA, pH 7.0:**
95 mg of EGTA (F.W = 380.4) in 10 ml water. pH with KOH.

**50 mM $CaCl_2$:**
74 mg $CaCl_2.2H_2O$ in 10 ml water

**Procedure:**

1. Inoculate FOUR 5 ml LB with MRE600 glycerol stock. Grow overnight at 37 ºC in shaker.

2. Inoculate TWO 500 ml LB with 5 ml of saturated, overnight MRE600 culture. Grow at 37 ºC in a shaker until A650 is 0.5. (Takes approximately 2-3 hours)

3. Transfer to 500 ml centrifuge bottles.

4. Spin at 8000 RPM for 15 min at 4 ºC in BECKMAN JA10 rotor.

5. Resuspend each cell pellet in 15 ml of Wash Buffer. Spin as in step 4. Resuspend again in 15 ml of S-30 Buffer.

6. Pool all the resuspended cells in one centrifuge bottle. Spin at 8000 RPM for 15 min at 4 ºC in BECKMAN JA10 rotor. <u>You can store the cell pellets at this stage at –80 ºC</u>.

7. Weigh the cell pellet. Resuspend the cell pellet in S-30 Buffer + 10% glycerol on ice:
   Add 1.5 ml buffer per Gram of cells

8. Lyse the cells using a French Press.at 10,000 PSI into a red-capped ultra centrifuge bottle (BECKMAN Cat# 355618) on ice. (Note: Before use, rinse the French press cylinder with water, then with S-30 buffer and cool the cylinder to 4 C)

9. Centrifuge at 20,000 RPM for 30 min at 4 C in Ti70 rotor.

10. Transfer the supernatant to a new ultra centrifuge bottle (discard the pellet). Centrifuge again at 20,000 RPM for 30 min at 4 C in Ti70 rotor.

11. Pool the supernatant and measure the A260, which should be about 150-250 units/ml. (Save about 20 ul at -80 C for assay).

12. <u>Nuclease Treatment:</u>
Dissolve 15,000 units of Micrococcal nuclease (Boehringer cat# 107921) in 200 ul of buffer (50 mM glycine-KOH, pH 9.2; 5 mM $CaCl_2$).

To 1 ml of S-30 supernatant from step 11, add 20.5 ul of 50 mM CaCl2 (final conc 1 mM) and 10 ul of Micrococcal nuclease (750 units). <u>Incubate at 30 C for 20 min.</u> (Note: I recovered 5.5 mls of S-30, so added 113 ul of 50 mM CaCl2 and 55 ul of nuclease).

13. Inactivate the nuclease by adding 86.65 ul of 25 mM EGTA-KOH, pH 7.0 (final conc 2 mM). (save 20 ul for assay). (I added 440 ul of 25 mM EGTA-KOH).

14. Centrifuge at 50,000 RPM for 2 hours at 4 C in Ti70 rotor. Note: The tube will collapse at 50K unless it contains 16 ml minimum volume.

15. Remove the supernatant. Dialyze against S-30 Buffer + 10% glycerol. Use 6-8K cut-off dialysis tubing. Dialyze TWO times against 100 volume for 45 minutes each at 4 C.

16. Make 50 ul aliquots and store at -80C. (About 100 aliquotes)