**Making protein lysates**

1. Thaw an aliquot of 4X SDS-PAGE loading buffer. Calculate the amount of 1X loading buffer you will need (~100 μl per sample, plus a little extra to account for pipetting error), then make that amount by diluting 4X SDS-PAGE buffer 1:3 with dH2O. The composition of the loading buffer is.

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| --- | --- |
| **Amount for 10 ml stock** | **Final concentrations (10X)** |
| 2.0 ml 1 M Tris-HCl pH 6.8 | 50 mM Tris-HCl pH 6.8 |
| 0.8 g SDS | 2% SDS |
| 4.0 ml 100% glycerol | 10% glycerol |
| 0.4 ml 14.7 M β-mercaptoethanol | 1% β-mercaptoethanol |
| 1.0 ml 0.5 M EDTA | 12.5 mM EDTA |
| 8 mg bromophenol blue | 0.02% bromophenol blue |
| dH20 to 10 ml |  |

2. Harvest 2E7 cells by centrifugation at 1000 rcf for 5 min. Turn on heat block set to 100 °C. Add water to wells of heat block to ensure even heating of sample.

3. Wash 1X with 1 ml 1X PBS. Place the tube of diluted 1X loading dye in the heat block for ~5 min to pre-heat. Note: may want to pierce the lid of the tube to relieve pressure during heating.

4. Remove all but ~20 μl of the supernatant.

5. Resuspend pellet in residual liquid. Pierce lid of tube.

6. Add 200 μl of pre-heated 1X loading buffer to each pellet. Flick gently to mix. Place each sample in heat block and incubate for 5 min.

7. Vortex each sample vigorously for 1 min to shear genomic DNA and make the sample easier to load.

8. Centrifuge sample for 1 min at maximum speed.

9. Transfer sample to a fresh tube (with unpierced lid). Label tube carefully and store at -20 °C until ready to analyze by SDS-PAGE.