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Preparing whole cell samples for immunoblot analysis

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Protocol status: Working

We use this protocol and it's working

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Abstract

Protocol for preparation of HeLa cell lysates for immunoblot analysis.



- 1 Add an appropriate volume of 1x LDS Sample Buffer (ThermoFisher) to each sample.
- 2 Boil each sample at 99 °C with shaking at maximum speed for 00:10:00 10m
- 3 Allow all samples to cool to Room temperature , and quickly centrifuge the samples to collect all liquid in the bottom of the tube. Vortex each sample for ~3 seconds to ensure homogeneity.
- 4 Measure the concentration of each sample spectroscopically, blanking with the 1x LDS sample buffer and using an A280 measurement. Dilute samples with 1x LDS Sample buffer to a concentration of < 7 mg/mL if required.
- 5 Aliquot out the desired amount of each sample into a separate tube, and add 1x LDS Sample Buffer to each sample to make all samples in the same gel a standard volume.
- 6 Either freeze samples at -20 °C until required, or load directly onto a SDS-PAGE gel.