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We use this protocol and it's working

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Preparing whole cell samples for immunoblotting

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ABSTRACT

Protocol for preparation of HeLa cell lysates for immunoblotting

Keywords: ASAPCRN

1 Add an appropriate volume of 1x LDS Sample Buffer 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.