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www.histonecode.com

Protein Lysate

Revised by J Sims
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Procedure

1. Collecting cells: Trypsinize cells off of petri dishes and spin down 500xg 5 min
2. Resuspend pellet in PBS and respin to wash out all media
3. Resuspend the cell pellets in 2x Laemmli to lyse the cells (final concentration of 10^7 cells/mL).
4. Boil the lysate for 10 minutes and homogenize using a 21-gauge needle (10-15x) after lysate has cooled.
5. For western blot analysis, we usually load the gels by cell number/lane using $\sim 10^5$ cells/lane or $\sim 10\mu\text{L}$ /lane.

In order to ensure that there is equal loading of the samples and to be able to repeat the results, it would be great to have enough lysate to repeat the blots several times (at least twice).

Solutions

2x Laemmli Buffer

0.2% SDS

3mM Tris

0.4M glycine

