Lysis Protocol for mammalian cell lines

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Reagents & Solutions

Component	Order number	Stock solution
HEPES	Aldrich H23830	prepare 200mM solutions at pH values of 8 and 8.5 using sodium hydroxide for adjustment.
Sodium-dodecyl sulfate (SDS)	Merck 05030-500ML-F	20% solution
cOmplete Protease Inhibitor Cocktail, EDTA free	Roche 05892791001	Prepare working solution as directed by manufacturer instructions.
Benzonase	Merck 71206-3	≥250 units/µL
Magnesium chloride	Merck 105833	Prepare a 1 M stock solution

Lysis buffer:
50mM HEPES (pH 8.5)
1% SDS
1mM $MgCl_2$
1X Protease Inhibitor Cocktail

Cell Lysis and Protein Preparation

1. Resuspend the cell pellet with lysis buffer (use two-three times the volume of cell pellet) and pipette mix briefly. **Critical**: Cells will lyse almost instantaneously with the addition of the lysis buffer. Pipetting should be minimized to avoid sticking of chromatin to the pipette tip.
2. Incubate the mixture at 95°C for 5 minutes and afterwards place the tubes on ice to cool.
3. Add 2 µL of benzonase per 1,000.000 cells and incubate for 30 minutes at 37°C.
   Note: The viscosity of the solution should be reduced after the incubation with benzonase compared to before, check by pipetting. If this is not the case, repeat this step or add a sonication step.
4. Centrifuge to get rid of cell debris (5000xg, 5min).
5. Transfer supernatant into fresh tube.
6. Perform protein determination assay.

The lysate can be frozen at this point and stored until use.

Quality control:
An SDS PAGE gel can be used to compare lysis protocols and to check the lysis efficiency. Aim to load around 5-10 µg. Gels should be visualized with colloidal Coomassie.