LYSING CELLS FOR NUCLEAR PROTEINS

Wash 10 cm dish of cells with PBS.

Add 0.5 ml of Lysis Buffer, leave plates on ice for 5 minutes and then vigorously scrape bottom of dish with clean cell scraper.

There are two methods we use to shear the DNA in the lysates. One is to transfer the lysate to an Eppendorf tube and syringe the cells repeatedly up and down with a 21G hypodermic. As a final step, spin down hard to pellet cellular debris and transfer the lysate to a new Eppendorf tube.

An easier alternative (and highly recommended for radioactive samples) is to spin the lysate through a QIAGEN QIAshredder column. Lysates with high protein/DNA content may require passage through more than one QIAshredder column, or a combination of shredding and syringing. Transfer the lysate to an Eppendorf tube when done, taking care to avoid any pellets that may be at the bottom of the tube.

Store lysates on ice to use the same day, or snap-freeze in liquid nitrogen for storage at –80C (when ready to use frozen samples, thaw quickly at 37C and transfer immediately to ice).

Nuclear Lysis Buffer:

Final Concentration	Stock Solution	Amount for 10 ml
50 mM Tris, pH 7.5	1 M Tris, pH 7.5	0.5 ml
0.5 M NaCl	5 M NaCl	1 ml
1% NP-40	10% NP40	1 ml
1% DOC	10% DOC	1 ml
0.1% SDS	5% SDS	0.2 ml
2 mM EDTA	0.5 M EDTA	0.04 ml
COMPLETE protease inhibitors	Mini Tablets (for 10 ml)	1 tablet
DH2O		Up to 10 ml