Protocol for isolating both cytoplasmic and nuclear extracts:
Solutions:

Harvest buffer:	For 50ml:
10mM HEPES pH 7.9	0.5ml 1M HEPES
50mM NaCl	0.5ml 5M NaCl
0.5M Sucrose	8.56g sucrose
0.1mM EDTA	10μl 0.5M EDTA
0.5% Triton x 100	2.5ml 10% triton

*freshly add to harvest buffer just before use:
1mM DTT
1mM PMSF
4 μg/ml Aprotinin
2 μg/ml Pepstatin A
10mM tetrasodium pyrophosphate (phoshphatase inhibitor)
100mM NaF (phoshphatase inhibitor)
17.5 mM β-glycrophosphate (phoshphatase inhibitor)

Buffer A (optional):	For 50ml:
10mM HEPES pH 7.9	0.5ml 1M HEPES
10mM KCL	250μl 2M KCL
0.1mM EDTA	10μl 0.5M EDTA
0.1mM EGTA	10μl 0.5M EGTA

*freshly add to buffer A just before use:
1mM DTT
1mM PMSF
4 μg/ml Aprotinin
2 μg/ml Pepstatin A

Buffer C:	For 50ml 1X:	**for 50ml 2X**
10mM HEPES pH 7.9	0.5ml 1M HEPES	1ml 1M HEPES
500mM NaCl	5ml 5M NaCl	10m 5M NaCl
0.1mM EDTA	10μl 0.5M EDTA	20μl 0.5M EDTA
0.1mM EGTA	10μl 0.5M EGTA	20μl 0.5M EGTA
0.1% (NP40)	250μl 20% NP40	500μl l 20% NP40

*freshly add to buffer C just before use:
1mM DTT
1mM PMSF
4 μg/ml Aprotinin
2 μg/ml Pepstatin A

Ice cold PBS and PBS-1mM EDTA

Method:

•Place plates on ice.
•Wash 2X with cold PBS-5mM-EDTA.
•Add 1ml PBS-5mM-EDTA and scrape cells. Transfer to 1.5ml tube.
•Pellet at 3000rpm 5 min.
•Resuspend in 50-500μl (2-4 vol) Harvest buffer.
•Incubate on ice 5min.
•Pellet at 1000rpm in a table top swinging bucket rotor (for example by resting microfuge tubes in 15ml tubes while spinning) for 10min to pellet nuclei @ 4C (preferable). Alternatively spin in a fixed angle rotor at 2000 rpm (425rcf). Note that the nuclei sometimes smear up the side of the tube in the fixed angle rotor.
•Transfer sup to new tube. For best results, clear at 14,000rpm for 15 min. & transfer sup to new tube = Cytoplasmic/membrane extract.
Save for PERK or IRE IP or eIF2α & GADD34 western.
•Wash/resuspend nuclear pellet in 500ul buffer A (optional) or harvest buffer.
•Pellet at 1000rpm in swinging bucket rotor 5min 4C.
•Remove and discard sup.
•Add 4vol buffer C. (**For a more concentrated extract use 2 vol 2X buffer Cs**.*)*
•Vortex 15min at 4C. (in cold room) Start on high speed vortex to loosen pellet,
then turn to medium.
•Pellet at 14,000rpm 10min 4C. For better recovery and clarity clear at 100,000g for 30min at 4C in a tabletop ultra (eg TLA100, or TLA55 in an Optima table top ultra centrifuge).
•Transfer sup to new tube. =Nuclear extract
If nuclear extract will be used for functional assays such as gel shift (EMSA), then 10% glycerol should be added prior to freezing.

Measure protein concentrations of cytoplasmic and nuclear extracts.
Load ~40-50μg of nuclear extract for XBP , ATF4 and CHOP western (20μg is enough for many cell types, but loading more is better.)
Load 25-40μg of cytoplasmic extract for P-eIF2alpha western.
Use 500-1500μg of cytoplasmic extract for PERK or IRE IP-western.
(The more the better.)

For the fixed angle rotor we use the eppendorf 5417R centrifuge with a 30 slot rotor. Below are RPM/RCF equivalents for our common speeds:

RPM	RCF
14000	20817
7000	5204
3000	956
2000	425
1000	106

For the swinging bucket rotor (also eppendorf):

RPM	RCF

1000	58
1500	131
2000	233

chemical	Mm Final	Formula	MW	mg/ml	g for 2L
tetrasodium pyrophosphate	10	Na4P2O7 · 10H2O	446.06	4.5	8.9
NaF	100	NaF	41.99	4.2	8.4
β-Glycerophosphate	17.5	CH2OHCH(OPO3Na2) CH2OH·5H2O	306.11	5.4	10.7
Amt (mg) of powdered mix to add per ml				14.0	

Notes*
1. Mix the powdered amounts for 2L in a 50 ml tube and store.
When needed, weigh out 14mg/ml * the solution volume and mix. The made up solution is good at 4C for up to 1 month.

2. These chemicals are available in various hydration forms. Make sure to use the one indicated or recalculate