PROTEIN PURIFICATION

Sections:

I. Buffer Preparation

a. 1X PBS pH 7.4
   i. Dilute 10X stock then check pH

b. Lysis Buffer (50mL)
   i. 500mM NaCl
   ii. 50mM HEPES
   iii. 10% glycerol
   iv. 1% Triton x-100

Component	**Amount**
Sodium Chloride	1.461g
HEPES	595.75mg
Glycerol	5mL
Triton x-100	500uL

c. Equilibration Buffer (50mL; pH 8.0)
   i. 100mM Sodium Phosphate (dibasic)
   ii. 600mM Sodium Chloride
   iii. 0.05% tween -20
   iv. 30mM Imidazole

Component	**Amount**
Sodium Phosphate Dibasic	1.34g
Sodium Chloride	1.7532g
1% Tween-20	2.5mL
1M Imidazole	1.5mL

d. Wash Buffer (50mL; pH 8.0)
    i. 100mM Sodium Phosphate (dibasic)
    ii. 600mM Sodium Chloride
    iii. 15% Glycerol
    iv. 0.05% tween-20
    v. 50mM Imidazole

Component	**Amount**
Sodium Phosphate Dibasic	1.34g
Sodium Chloride	1.7532g
Glycerol	7.5g
1% Tween-20	2.5mL
1M Imidazole	2.5mL

e. Elution Buffer (50mL; pH 8.0)
    i. 100mM Sodium Phosphate (dibasic)
    ii. 600mM Sodium Chloride
    iii. 15% Glycerol
    iv. 250mM Imidazole

Component	**Amount**
Sodium Phosphate Dibasic	1.34g
Sodium Chloride	1.7532g
Glycerol	7.5g
1M Imidazole	12.5mL

f. When making these buffers, please fill with H2O to ~40mL. Then pH balance before adding the remaining water.
g. 1M Imidazole
    i. 3.3g/50mL
h. 1% Tween-20
    i. 500uL tween-20 + 49.5mL H20

DAY 1

II. Cell Isolation

a. Dislodge the cells from the surface of the plate by using the media found within the plate.
b. Transfer the cells and media into a 50mL conical
    i. Keep Tube on Ice from this Point On
c. Pellet the cells
    i. 500g; 5 min; 4°C
d. Remove the supernatant
e. Resuspend in 10mL COLD 1X PBS
    i. Resuspend via pipetting
f. Transfer into a 15mL conical
g. Pellet
    i. 500g; 5min; 4°C

III. Cell Lysis

a. Please move into the Cold Room at this Point
    i. When working with multiple plates, it is best to work in batches.
    ii. Best practice is to only Lyse 2 tubes at a time
    iii. Keep one batch of tubes in ice while you Lyse the other batch. Do not remove the supernatant/PBS from these tubes.
b. Remove the PBS from the tubes
c. Resuspend in 2mL of Supplemented Lysis Buffer
    i. Resuspend by pipetting
    ii. For 1mL of Supplemented Lysis Buffer
        i. 1mL Lysis Buffer
        ii. 10uL 100X HALT
        iii. 1.6uL 300mM PMSF
d. Pass the solution through a P1000 pipette, then 15G needle, 20G and 23G needle to shear the DNA for 15 min
    i. You should alternate the shearing between the two tubes, placing them on ice in between.
    ii. You should aim to pass the solution through the needle 3 times within the 15 min.
e. Transfer into 2mL tubes
    i. 4 tubes per sample/plate
    ii. 500uL per 2mL tube
    iii. Collect 10uL for gel
f. Centrifuge
    i. 10,000g; 10min; 4°C
    ii. Centrifuge should be available within the cold room
    iii. Lysis of other cells can be done during this time

g. Transfer the Lysate to a new batch of 2mL tubes
    i. Save 10uL for a gel
h. Add enough supplemented Equilibration Buffer to create a 1:1 solution; Lysate/Equilibration Buffer and mix.
    i. Buffer Supplemented to 1X HALT
    ii. The tubes are safe to leave on ice at this point if you wish to continue lysing the other cells.
    iii. Should only be left for less than half an hour.
i. Beads can be prepared at RT at this point
    i. 50uL of well mixed beads
        1. Total of 100uL of beads per plate of cells
    ii. Add 200uL of UNSUPPLEMENTED Equilibration buffer
    iii. Vortex 10 seconds
    iv. Magnet
    v. Discard supernatant
    vi. Remove from magnet
    vii. Add 500uL UNSUPPLEMENTED Equilibration Buffer
    viii. Vortex 10 Seconds
    ix. Magnet
    x. Bring to cold room and remove supernatant.
j. Add the whole lysis mixture to the 50uL of beads
k. Place on a rotator for overnight at 4°C

<u>DAY 2</u>

l. Liquid Nitrogen will be needed for the following steps
m. Place your tubes on the magnet
    i. Collect 10uL for gel “FT1”
    ii. Freeze the remaining FT in liquid Nitrogen
n. Remove the tubes from the magnet
o. Wash in 500uL supplemented Wash buffer
    i. Supplemented with 1X HALT
    ii. Resuspend by pipetting
p. Place on magnet
    i. Collect 10uL for gel “FT2”
    ii. Freeze the remaining FT in liquid Nitrogen
q. Remove from magnet
r. Wash with 500uL supplemented wash buffer
    i. Resuspend by pipetting
s. Combine the tubes with their corresponding plates

   i. You should have ~1mL in each tube afterwards
t. Place on magnet
   i. Collect 10uL for gel “FT3”
   ii. Freeze the remaining FT in liquid Nitrogen
u. Remove from magnet
v. Resuspend in 125uL supplemented elution buffer
   i. Supplemented to 1X HALT
   ii. 150ng/uL 3X FLAG peptide
   iii. 15% Glycerol
   iv. Rest is TBS
w. Rotator for 30 min
x. Place on the magnet
   i. Collect 10uL for gel
   ii. Freeze remaining elution in liquid Nitrogen
y. Save beads to run on gel.