Title: Preparation of protein lysates from FFPE tissues.

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Revision History

Revision date	Revision Author	Revision notes

I. Chemical Reagents and Recommended Sources

1. Xylene (Acros, 422685000)
2. Ethanol (EtOH) (Decon Labs, 2701)
3. Ammonium bicarbonate (NH4HCO3) (Fisher, A643-500)
4. RapiGest SF surfactant (Waters, 186001861)
5. Water, HPLC grade (Fisher, W5-1)
6. Phosphatase Inhibitor Cocktail 3 (Sigma, P0044)
7. Phosphatase Inhibitor Cocktail 2 (Sigma, P5726)
8. Protease Inhibitor Cocktail (Sigma, P8340)
9. pH strips (MColorpHast 1.09535.0001, range 0-14)

II. Reagent Preparation for FFPE Cell Lysis and Protein Digestion

A. 50mM NH4HCO3, pH 8.0. **Must be made fresh weekly**:
1. Add at least 10mg NH4HCO3 for every 5 samples to be processed to a 15mL Falcon tube.
2. Add HPLC water to a final NH4HCO3 concentration of 3.953mg/mL.
3. Mix well.
4. Test pH should be 8.0 by paper test strip.
5. Label and store at 4°C.

B. 50mM NH4HCO3, pH 8.0 with inhibitors. **Must be made fresh weekly**:
1. Transfer at least 1mL 50mM NH4HCO3 for every 10 samples to be processed to a labeled 15mL Falcon tube.
2. Add 1% Phosphatase Inhibitor Cocktail 3.
3. Add 1% Phosphatase Inhibitor Cocktail 2.
4. Add 1% Protease Inhibitor Cocktail.
5. Mix well.
6. Label and store at 4°C.

C. 0.2% (w/v) RapiGest in 50mM NH_4HCO_3, pH 8.0. **Must be made fresh daily**:
1. To one 1mg vial of RapiGest, add 500μL of 50mM NH_4HCO_3, pH 8.0 with inhibitors.
2. Mix until dissolved.
3. Store on wet ice until use.

V. Deparaffination, Rehydration and Resolubilization Protocol of FFPE Samples

A. Deparaffination with xylene **(Repeat 3x)**
1. Place slides in gray 24 slide holder.
2. Add 150mL xylene to three tubs. Xylene may be recycled from previous experiments.
3. Place slides in tub for 3 min.
4. Drain & blot excess solution and move slides to next tub.

B. Rehydration of FFPE samples
1. Add 150mL 100% EtOH to two tubs. 100% EtOH may be recycled from previous experiments.
2. Place slides in tub for 3 min.
3. Drain & blot excess solution and move slides to next tub.
4. Add 150mL 85% EtOH to two tubs. 85% EtOH may be recycled from previous experiments.
5. Place slides in tub for 3 min.
6. Drain & blot excess solution and move slides to next tub.
7. Add 150mL 70% EtOH to a tub and 150mL H_2O to another tub. 70% EtOH and H_2O must be fresh.
8. Place slides in tub for 3 min.
9. Drain & blot excess solution and move slides to next tub.

C. Resolubilization
1. Add 100 µL 0.2% RapiGest in 50 mM NH_4HCO_3.
2. Incubate at 95 °C with mixing at 1000 rpm for 30 minutes.
3. Cool on ice for 5 min.
4. Sonicate 3x in cup horn probe (filled with ice water) at 50% power for 30 s, incubate on ice for at least 30 s between each sonication step.
5. Incubate at 80 °C with mixing at 1000 rpm for 120 minutes.
6. Cool on ice for 5 min.
7. Add 100 µL 50 mM NH_4HCO_3, pH 8.0.
8. Sonicate 3x in cup horn probe (filled with ice water) at 50% power for 30 s, incubate on ice for at least 30 s between each sonication step.

D. Storing samples until digestion
1. Cool samples on ice for 5 min.
2. Store samples at -80°C until the day of digestion.
3. On day of digestion, thaw samples on ice for 5 min.
4. Vortex samples for 10-15 s.
5. Centrifuge at 20000 rcf for 10-20 min at 4°C.