Body Fluid Identification by Proteomic Mass Spectrometry - Digestion

1 Purpose

1.1 Overnight digestion of samples for Body Fluid Proteomics Assay to identify specific body fluids on evidence samples using liquid chromatography and mass spectrometry.

2 Protein Digestion Day 1 Procedure

2.1 Input Concentration Avg values from Quant Batch into Digestion Batch in LIMS.

2.2 Ensure copy and paste from Quant batch into Digestion Batch was done correctly.

2.3 Retrieve sample Extraction tubes and positive control extracts from -20°C.

2.4 **Label WITNESS**: Have a witness verify that correct tubes are present in the set and the labels on input tubes match labels on output tubes, and that the ENEG volume is 50 µl.

2.5 Pipette volumes calculated (10 µg protein for non-LOW QUANT samples) by the LIMS into new 1.5 microcentrifuge Digestion tube.

- Note: Always transfer 50 µl of Eneg extract.

2.6 For LOW QUANT samples (low concentration i.e., those with < 0.2 µg/µl) transfer total sample volume. Record sample volume in LIMS.

2.7 Place non- LOW QUANT extraction tubes in -20°C freezer.

2.8 Turn on both Mini-Shakers and set to 37°C and 55°C respectively.

2.9 Retrieve the 250 mM TCEP solution from freezer (-20°C) and record the identification number in LIMS. Add a tick mark to microcentrifuge tube; do not allow more than 5 freeze-thaw cycles. Place on ice or in a 4°C cold tube rack.

2.10 Add 1 µl of 250 mM TCEP into all tubes. Vortex and short spin on benchtop centrifuge.

2.11 Incubate for 30 minutes on the Mini-Shaker at 55°C at 200 RPM. Record instrument and temperature in LIMS.

2.12 Retrieve the 500 mM IAA from freezer (-20°C) and record the identification number in LIMS. Add a tick mark to microcentrifuge tube; do not allow more than 5 freeze-thaw cycles. Place on ice or in a 4°C cold tube rack.

2.13 Remove sample tube from heating Mini-Shaker incubator and let cool to room temperature for five minutes.

2.14 Add 1.5 µl of 500 mM IAA into all tubes. Vortex and short spin on benchtop centrifuge.

2.15 Incubate in the dark (in drawer) at room temperature for 20 minutes.

2.16 Retrieve 500 mM DTT from freezer (-20°C) and record the identification number in LIMS. Add a tick mark to microcentrifuge tube; do not allow more than 5 freeze-thaw cycles. Place on ice or in a 4°C cold tube rack.

2.17 Add 1.5µl of 500 mM DTT into all tubes. Vortex and short spin on benchtop centrifuge.

2.18 Incubate at room temperature for 20 minutes (on the bench, darkness not required).

- Note: Sample may or may not turn opaque, continue regardless.

2.19 Retrieve 0.5 µg/µl trypsin from freezer (-80°C) and record the identification number in LIMS. Add a tick mark to microcentrifuge tube; do not allow more than 5 freeze-thaw cycles. Place on ice or in a 4°C cold tube rack.

2.20 Add 1µl of 0.5 µg/µl trypsin into all tubes. Vortex and short spin on benchtop centrifuge.

2.21 Incubate at 37°C in Mini-Shaker overnight (17±1 hours) at 200 RPM. Record instrument and temperature in LIMS.

3 Protein Digestion Day 2 Procedure

3.1 Retrieve the following reagents and consumables, record the lot and identification numbers into LIMS.

Formic Acid (FA) at 4°C
Breathe-easy breathable tube membrane

3.2 Remove tube from incubator and quick spin on benchtop centrifuge for 5 seconds to pellet droplets on lid.

3.3 Add 2.8 µl of formic acid to all tubes and vortex.

3.4 Spin tube in benchtop centrifuge at 12,000 g for 15 minutes.

3.5 **Transfer Witness:** Witness tube top labels and LIMS labels match.

3.6 Transfer the following volumes into new 1.5ml microcentrifuge tubes:

3.6.1 **REGULAR Samples**: Transfer 50 µl of the digested supernatant. Quick spin on benchtop centrifuge.

3.6.2 **LOW QUANT Samples** Transfer total sample volume. Quick spin on benchtop centrifuge.

3.7 Place Breathe-Easier Breathable Tube Membrane on top of open tubes.

3.8 Dry sample in SpeedVac (no temperature setting required) until samples are dry. MUST check if sample is dry in 1-hour increments.)

3.9 Remove Breathe-Easier Breathable Tube Membranes and store dried digestion tubes at -20ºC.