

## MOLECULAR SEROLOGY PROCEDURES MANUAL

<b>Body Fluid Identification by Proteomic Mass Spectrometry -Digestion</b>		
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### 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.
- 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.

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- 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.

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- 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  $\mu$ l 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 -20°C.

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