

Tips for Active Neutrophil Serine Protease Analysis:

Since many proteases are autolytic and have very short life spans, special care needs to be taken when measuring active NSPs vs total protein in sputum samples. Below are some considerations and tips for processing and handling lung samples for protease analysis as a biomarker.

1. Keep samples as COLD as possible
 - If possible, samples from the lung should be stored or transported on ice from the moment of collection.
 - Frozen sample should be thawed slowly and kept on ice.
 - Aliquot sample into small amounts for single use after thawing.
 - Keep sample on ice while carrying out the assay.
2. Use appropriate tools
 - Each type of sample may require any of a variety of tools including tweezers, forceps, scalpels, scissors, petri dishes, etc.
3. Avoid reducing agents
 - Reducing agents (like DTT or Sputolysin) may lead to inaccurate results
 - Split sample prior to adding reducing agent so that untreated sample can be analyzed for protease activity and compared to treated until it is established that reducing agent does not alter values in the protocol

Suggested Sputum Processing Protocol for Protease Biomarker Analysis:

1. Upon collection, place sputum on ice
2. Transfer sample to 50mL tube
3. Weigh sample
4. Add 4 parts ice cold PBS
5. Invert tube 10x (DO NOT Vortex)
6. Pre-chill centrifuge to 4°C
7. Centrifuge sputum for 30 minutes at 3,000g at 4°C
8. Carefully remove supernatant with a pipette to a new tube
9. Continue to keep sputum sample on ice at all times
10. Divide into 225µL aliquots and store long term at -80°C until analysis

