FASP Kit Protocol - ORNL

Developed for Bacteriophage

This method was optimized for bacteriophage by Kristen Corrier undergraduate research assistant at Oak Ridge National Laboratory (ORNL), Chemical Science Division using the commercial method from Protein Discovery (Knoxville, TN) as a starting point.

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Dr. Nathan VerBerkmoes (ORNL, Chemical Science Division) oversaw the optimization process and creation of final method.

This method is designed to prepare small quantities (~1-10ug) of isolate bacteriophage or mixed bacterial/bacteriophage communities for shotgun proteomics via 2d-LC-MS/MS.

Please note this method is not effective for large quantities of material (>100ug), standard solution digest should be used for such samples. The minimum protein amount needed is estimated to be 500ng but this is highly sample dependent.

The main optimization of this method was to simplify it and greatly reduce the time required. This current method takes roughly half the amount of time (actually bench time) as the original commercial method, furthermore its now very straightforward to prepare 8, 16, or 24 samples at a time.

Urea/SDS Solution

1. In falcon tube, add 1 fleck DTT (10mM) to 5mL Tris HCl (provided with FASP kit) or Tris $CaCl_2$. Vortex briefly.
2. Add 1mL Tris buffer + DTT to 1 tube urea (75 µg, provided with FASP kit). Vortex until all powder dissolves.
3. Combine 300µL Urea solution and 150µL sample (sample can be in any form, mixture of viral proteins and bacteria, ionic or non-ionic detergents, buffers and/or CsCl).
4. Rock at room temperature for 30 min to 1 hr to lyse bacteria cells/phage particles

Protocol

Day 1

1. Transfer Urea solution + sample to spin filter. Centrifuge at 14,000 x g for 15 min.
2. Add 200µL fresh Urea solution (**no DTT, no SDS**). Centrifuge at 14,000 x g for 15 min.

    Urea solution:

    1mL Tris $CaCl_2$ or Tris HCl added to one tube of urea. Vortex until all powder dissolves.

3. Discard flow-through.
4. Add 10µL iodoacetamide solution and 90µL Urea solution (**no DTT, no SDS**). Vortex for 1 min, then incubate without mixing for 20 min in the dark.

    Iodoacetamide solution:

    100µL Urea solution (**no DTT, no SDS**) added to 1 tube iodoacetamide (provided with FASP kit). Pipette up and down 10-15 times to mix well and dissolve.

5. Centrifuge at 14,000 x g for 10 min.
6. Add 100µL Urea solution (**no DTT, no SDS**). Centrifuge at 14,000 x g for 15 min. Repeat this step twice.
7. Discard flow-through.
8. Add 100µL 50mM ammonium bicarbonate solution (provided with FASP kit). Centrifuge at 14,000 x g for 15 min.
9. Transfer filter to new collection tube.
10. Add 75µL digestion solution. Vortex briefly. Incubate at 37°C for 4 – 18 hours (NO ROCKING)

    Digestion solution:

    75uL ammonium bicarbonate solution added to 20µg trypsin (1 tube).

Day 2

11. Add 40µL 50mM ammonium bicarbonate solution. Centrifuge at 14,000 x g for 10 min. Repeat this step once.
12. Add 50µL 0.5 M sodium chloride solution (provided with FASP kit). Centrifuge at 14,000 x g for 10 min.
13. Filtrate contains digested proteins. Add 170µL* H_2O + formic acid. Aliquot (150µL x 2)*, freeze.

    *Volumes may be adjusted. Final filtrate volume = 130µL

    *Generally two replicates can be obtained from one filter

    *Sample is now ready for 2d-LC-MS/MS, remember a on-line desalting step is needed before SCX salt pulses start.