S-Trap sample processing: urine

S-Trap sample processing works well with urine and it must first be concentrated. The addition of SDS to urine during concentration prevents protein loss to centrifugal concentrators. Researchers must bear in mind that the protein content of urine is highly variable and depends on health and hydration state. If a protein assay (BCA) is not run before sample preparation, peptide levels after digestion must be matched (e.g. use 3-(4carboxybenzoyl)quinoline- 2-carboxaldehyde (CBQCA) or o-phthalaldehyde (OPA) assays).

Use the appropriate S-Trap sizes: micros are good for 100 µg max, minis for 300 µg max. The below is designed for 100 µg using a micro. Volumes and amount of protein processed may be scaled.

Protocol

1) Aliquot sufficient urine for 100 µg total protein. If the urine is at 10 mg/dL, this is 1 mL. Volume should be scaled accordingly and may greatly exceed 1 mL for highly dilute urine samples.

2) Add 12.5 µL of a 10% SDS stock solution to the sample. The SDS serves the function of 1) preventing protein loss on the centrifugal concentrator; 2) denaturing and stabilizing the proteins (especially during reduction and alkylation); and 3) facilitating S-Trap protein processing, which requires SDS.

3) Load SDS plus urine onto a centrifugal filter (not provided) and concentrate to 25 µL. We recommend the Vivaspin® 2 Centrifugal Concentrator (3 kDa cutoff, any membrane material). Additional urine may be added to filter as needed; do not add additional SDS if additional urine is added. The SDS micelles will not pass through the membrane. If the volume is too little (meaning the concentration has gone too long), simply bring the volume up to 25 µL with buffer (PBS, TEAB or tris). Concentration will result in a 25 µL of sample containing 100 µg protein in 5% SDS (final concentration).

4) Take the 25 µL and process per S-Trap micro protocol: reduce, alkylate, acidify, add S-Trap binding buffer, wash, digest and elute peptides.