SP3 Protocol

Version: March 2018

Materials

1. SeraMag Speed Beads – the 15mg bead stocks can be purchased commercially (GE Healthcare, CAT#45152105050250, CAT#65152105050250).
2. 100% ethanol – absolute ethanol can be sourced from a variety of vendors. Do not use denatured ethanol.
3. 80% ethanol – stock solution prepared using absolute ethanol.
4. Magnetic rack capable of holding 1.5mL tubes.
5. 0.2M HEPES, pH 8 – stock solution prepared from Sigma, CAT#H3375. Adjust pH using a solution of 2N NaOH prepared from Sigma, CAT#S8045.
6. Trypsin + rLysC mix – Promega, CAT#V5073.
7. Bath sonicator – this item is optional, but will improve protein elution from the SP3 beads.

Method

Processing from this point assumes the use of 100µg of protein material from each of the lysates prepared above. The described workflow is compatible with a range of input amounts, and should be scaled based on the requirements of the samples in question.

1. Set a Thermomixer to 24°C heating and 1,000rpm mixing.
2. Prepare a working solution of SP3 beads based on the number of samples to be processed (see Note 1).
3. Add an appropriate amount of the SP3 bead stock to 100µg of protein from each of the cell lysates. Pipette mix to homogenize the beads and lysate.
4. Add 100% ethanol to achieve a final proportion of 50% by volume (50% ethanol final concentration) (e.g. for 100µL of lysate + beads, add 100µL of 100% ethanol). Pipette mix briefly to homogenize the beads, lysate, and ethanol (see Note 2).
5. Place tubes in the Thermomixer and incubate at room temperature for 10-minutes with shaking at 1,000rpm.
6. Place tubes in a magnetic rack and incubate for 2-minutes or until the beads have settled to the tube wall.
7. Remove and discard the supernatant taking care not to disrupt the beads.
8. Remove the tubes from the magnetic rack and add 200µL of 80% ethanol. Gently pipette to reconstitute and rinse the beads.
9. Place the tubes on the magnetic rack and incubate for 2-minutes or until the beads have settled to the tube wall.
10. Remove and discard the supernatant.

11. Repeat Steps 8 – 10 two further times.
12. Using a 200µL pipette and tip, remove as much ethanol from the tubes as possible (see Note 3).
13. Prepare sufficient digestion solution for your samples (see Note 4).
14. Add 100µL of 50mM HEPES pH 8 containing trypsin/rLysC (1:25 enzyme:protein (µg/µg)) to each tube. Using the tip of the pipette, gently push the beads from the side of the tube wall into the liquid. Do not try to pipette the liquid (see Note 5).
15. Sonicate the tubes in a bath for 15-seconds to reconstitute the beads.
16. Incubate tubes overnight (14-hours) at 37°C with mixing at 1,000rpm in a Thermomixer.
17. The next day, spin the tubes at 20,000g for 1-minute to pellet the beads.
18. Place on a magnetic rack for 2-minutes or until beads have settled to the tube wall.
19. Recover the supernatant to a fresh 1.5mL tube.
20. The peptides can be frozen and stored indefinitely at this point.

Notes

1. The SeraMag beads are provided at an approximate stock concentration of 15mg/mL. SP3 is generally performed at a minimum of 10:1 µg/µg of beads:protein. To prepare the stock solution for 100µg of protein, take 10µL of each bead stock and combine into a single tube. Place on a magnetic rack for 1-minute to pellet the beads, and remove the supernatant. Off the magnetic rack, reconstitute the beads in 500µL of water, and pipette mix. Place on the magnetic rack for 1-minute and remove the supernatant. Reconstitute the beads at a concentration of 20µg/µL in water. Scale for the number of samples to be processed.
2. Depending on the amount of protein present, the beads may clump and become very sticky immediately after the addition of ethanol. To avoid material loss due to beads sticking to the pipette tip, minimize the amount of post-addition pipetting that is performed.
3. Try to remove as much ethanol as possible with pipetting prior to digestion. It is not necessary to air-dry the beads prior to the addition of the digestion solution.
4. Choose the amount of digestion buffer based on your sample amount. In our hands, 100µL volumes of digestion solution provide a good balance between reconstitution and limited sample dilution.
5. Avoid pipette mixing the beads during reconstitution. Depending on how much material was processed with SP3, the beads may be very sticky, and material can be lost on pipette tips. It is recommended to add the digestion solution and simply push the beads into it using a pipette tip. Sonication in a water bath will break up the bead clump, which can then be pipetted.