Sample preparation for bottom-up proteomics analysis

Collect cells in an Eppendorf tube, or a Falcon tube in case the pellet is more than 50-100 µl. Make sure they have been washed with PBS (otherwise proteins from the media will interfere with the analysis).

Prepare lysis buffer as follows: 6 M urea, 2 M thiourea, 1X protease and phosphatase inhibitor cocktail.

Mix the minimum volume possible of lysis buffer with the cell pellet. You can start from about 100 µl. This is because we need to dilute the urea before digestion, and we end up with a huge volume if we include too much from the start. Pipette up and down to lyse the cells. The solution should become kind of viscous. You can freeze and thaw to assist cell lysis. Do not heat or sonicate, because urea reacts with proteins at high temperatures.

Tip: you can verify that the cells are lysed by centrifugating the solution (5,000 g for 1-2 min) and check whether the pellet became much smaller as compared to the initial one.

Dilute sample with 5 times the initial lysis buffer volume using the following buffer: 50 mM ammonium bicarbonate and 5 mM dithiothreitol (DTT). The solution should be at pH 8. You can check with a drop on a pH strip, but trust me, it is! Do not dip a pH meter inside the solution.

Leave the solution for 1 hour at room temperature. DTT is a reducing agent. This step opens all protein disulfide bonds, which will assist digestion. You should have a solution of about 600-700 µl at this point, if you started from 100 µl cell pellet. If you see a white filament in the solution, that is DNA. By not sonicating we did not break it. You can ignore it, but if it bothers you can add 1-2 µl of benzonase (not sure about the company, any will do the job), an enzyme that cleaves DNA to single nucleotides.

Add iodoacetamide to a final concentration of 20 mM, and incubate 30 min in the dark. Try to prepare concentrated iodoacetamide, so you can put a small volume in the final sample. Prepare iodoacetamide fresh right before the incubation, as it is a photosensitive reagent. This chemical derivatizes cysteine side chains, in order to avoid reformation of disulfide bonds even in not reducing environment. The concentration is more than double of DTT because it guarantees that all reactive DTT is derivatized as well. DTT has two reactive groups… plus all free cysteines… everything blocked!

If possible, quantify the protein amount using Bradford or similar strategies. Try to use as blank a solution containing the components you add to the mixture (both urea and iodoacetamide absorb a bit). Otherwise guess based on your experience ☺

Add trypsin at a ratio between 1:20 to 1:100 (w/w). For instance, 1 µg of trypin every 50 µg of sample. Incubate overnight at room temperature. This will digest proteins. Dry the sample the following day.

Reagent list

Urea (http://www.sigmaaldrich.com/catalog/product/sigma/u5378?lang=en&region=US)

Thiourea (http://www.sigmaaldrich.com/catalog/product/sial/t8656?lang=en&region=US)

Protease and phosphatase inhibitor cocktail (Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free (100X), catalog # 78445)

Ammonium bicarbonate (http://www.sigmaaldrich.com/catalog/product/sial/09830?lang=en&region=US)

Dithiothreitol (http://www.sigmaaldrich.com/catalog/product/sial/d0632?lang=en&region=US)

Iodoacetamide (http://www.sigmaaldrich.com/catalog/product/sigma/i1149?lang=en&region=US)

Trypsin (https://www.promega.com/products/mass-spectrometry/proteases-and-surfactants/trypsin-for-protein-characterization/trypsin-reagents/sequencing-grade-modified-trypsin/)