Mass Spectrometry

Cell Lysis Protocol

Cells lysis in 8M urea for mass spectrometric analysis

Before starting the cell lysis please contact our facility to discuss the optimal cell lysis procedure

Lysis Buffer: 200mM ammonium bicarbonate, 8M Urea

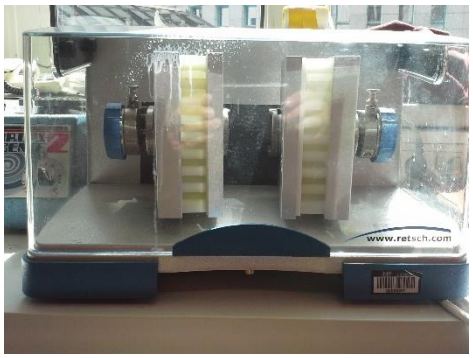
The cell pellet should be washed 3 times with PBS before the lysis.

The buffer is added directly to pellet. The amount of buffer which is used depends of the expected yield of protein. E.g. 1e6 cells (10cm dish) will contain at least 1mg of protein. The protein extract should be produced as concentrated as possible, because it has to be diluted for the subsequent digest to 2M Urea.

Break up the cells according to the method normally used in your group, but use the Urea-ABC buffer instead of the standard lysis buffer. Determine the protein concentration before submitting the sample to the MS facility.

Here we provide two examples of possible methods for cell lysis

Example nr1 for tissue samples: Use a tissue homogenizer (pic.1) with tungsten beads (pic2). The sample is shaked for a minute with one bead. Then the bead is removed and the sample is treated with the sonicator probe (pic 4) for one minute. Spin the sample at 15000 rcf with the Eppendorf centrifuge for 10 minutes. If a pellet is visible try to lyse it again with the sonicator probe (cycle: 1 Amplitude 100%, Pic 3). If it cannot be resolved take the supernatant for digestion.



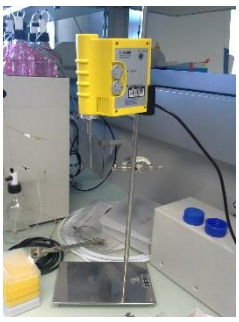
Picture 1: Tissue lyser II (Keays lab)

Picture 2: Tungsten bead in 2mL Eppendorf tube

Example nr2 for cell culture cells:

Add the buffer to the pellet and resuspend the pellet using a 27G needle and corresponding syringe. Subsequently use a 20G needle and resuspend the cell suspension for 10 times. Keep the suspension on ice for 5 minutes, then repeat the procedure. Spin the sample at 15000 rcf with Eppendorf for 10 minutes. If a pellet is visible try to lyse it with the sonicator probe (pic 4) for one minute (cycle: 1 Amplitude 100% pic 3). Leave the sample on ice for a minute and sonicate again. If it cannot be resolved take the supernatant for digestion

Picture 3: Sonicator probe with right settings



Picture 4: sonicator probe