

Mass Spectrometry-Appropriate Protocol for Cell Lysis using Probe Sonication.
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1. Wash cell pellet 3x with 10 volumes of 50 mM Ammonium Bicarbonate. Pellet cells gently between washes by centrifuging at ~5000 rcf for 3 minutes. Carefully pipette supernatant off after final wash, so as to not disturb the cells.
2. Add 5 volumes of lysis buffer, and vortex to suspend. The minimum volume for probe sonication is 100 uL in a 0.5 mL eppendorf tube. Standard lysis buffer for proteomics applications is 0.1%-0.25% w/v Rapigest SF (www.waters.com) in 50 mM Ammonium Bicarbonate. To increase coverage for membrane-bound and integral membrane proteins, substitute with 0.5% w/v PPS Silent Surfactant (www.proteindiscovery.com) in 50 mM Ammonium Bicarbonate.
3. Place samples on ice.
4. Perform probe sonication with 3 cycles of 15 seconds on, 5 seconds off, at 20% power. If possible, keep samples on ice during sonication to prevent excessive heating.
5. Centrifuge samples at 15,000 rcf for 5 minutes, and measure protein concentration of the supernatant using a mini Bradford (www.bio-rad.com) or micro BCA (www.piercenet.com) assay, depending upon the lysis buffer composition.