

Resource Summary Report

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A membrane-enriched preparation of culture samples for mass spectrometry-based proteomics

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Proper Citation

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Protocol Information

URL: <https://dx.doi.org/10.17504/protocols.io.bdapi2dn>

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Group: Coleman Lab

Summary: Purpose: Preparation of culture samples for mass spectrometry-based proteomics Principle: Utilizing a membrane-enrichment method of lysing cells and preparing peptides has yielded higher representation of membrane proteins in our mass spectrometry-based proteomic results. Traditional methods do not adequately extract or digest hydrophobic, transmembrane proteins. Particularly, we can now see full expression patterns of proteorhodopsin, something we could not detect using traditional mass spec proteomics prep. This protocol builds on the work of Molloy (2008) Methods Mol Biol (doi:10.1007/978-1-60327-064-9_30), Erde et al. (2014) J. Proteome Res. (doi:10.1021/pr4010019), and Waldbauer, et al. (2017) Anal. Chem. (doi:10.1021/acs.analchem.7b02752). Pure culture samples were spun down and flash frozen for proteomics. A carbonate extraction protocol was used for membrane enrichment before eFASP. The membrane fraction was enzymatically digested with both chymotrypsin and trypsin and the cytosolic fraction was digested with just trypsin. These samples were then ready to be processed further by in vitro isotopic peptide labeling (diDO-IPTL).

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