HYPERsol: flash-frozen results from archival FFPE tissue samples

Other

John Wilson (john@protifi.com), ProtiFi, LLC

Formalin-fixed, paraffin-embedded (FFPE) sample preservation is standard in all pathology departments where diagnosis is based on tissue section staining and immunohistochemistry. Uniquely, FFPE yields biologically inactive samples stable at room temperature for decades and longer, resulting in countless massive historical tissue archives worldwide. When paired with medical records describing the diagnosis and course of disease, these specimens represent an invaluable resource for biomedical research. However, both paraffin and crosslinking are incompatible with mass spectrometric proteomics analysis. Further, protocols for FFPE sample preparation lack standardization, efficiency, reproducibility and scalability. We reasoned that FFPE samples, inclusive of paraffin, could be substantially dissolved by a large excess of SDS in the presence of heat and megasonication, thus yielding proteomics results that closely mimic the original tissue.

In High-Yield Protein Extraction and Recovery by direct SOLubilization or HYPERsol, entire FFPE samples – without deparaffinization – are dissolved in 5% SDS with heating and 96-well plate PIXUL megasonication; samples are subsequently processed in 96-well plate S-Traps. This approach yields proteome coverage and quantification from FFPE samples very similar to that from paired flash-frozen tissue: peptide identification rates are 101% of flash-frozen, protein ID rates are 97% and the average correlation of protein quantifications is 0.936. Additionally, the approach recovers >2-fold more protein than traditional approaches, and protein can be directly quantified on S-Trap plate via BCA-no-more.

HYPERsol is an optimized, high-throughput workflow suited to the needs of biorepository analysis. It enables highly reproducible protein identification and quantification from FFPE tissue, yielding results that are highly similar to flash-frozen tissue. Direct resuspension of samples in 5% SDS obviates the need for deparaffinization, thereby increasing sample throughput and improving agreement with results obtained from paired flash-frozen tissue. We anticipate that the HYPERsol workflow will enable novel discoveries from rich clinically annotated and histologically characterized FFPE biorepositories worldwide.