

ProteoSolve-TD2 PrEP: Total Protein Recovery from Solid Ovarian Tumors With ProteoSolve-TD2 and PCT

Introduction

The classic method for the extraction of proteins from tissues is the Dounce homogenizer; however, this method was developed on soft, easily-disruptable, tissues, like liver (1-3). Many solid tumors, like ovarian, are composed of densely packed epithelial cells with connective tissue. These can be difficult to cut with a scalpel, and are impossible to lyse in a Dounce homogenizer. Mechanical homogenizers (rotor-stator or blender types) can be used on such tissues but require large volumes of fluid (> 10 mL) to operate. Mechanical homogenizers also tend to blend air into the sample and result in proteins lost to foam. Furthermore, the connective tissue present in solid tumors tends to shred into strings that wrap around the blades in mechanical homogenizers inhibiting their operation and making them difficult to clean between samples. ProteoSolve-TD2 and Pressure Cycling Technology (PCT) overcome these sample processing issues generating a concentrated protein sample suitable for 2-D gel analysis from tough to process tissues, like metastatic ovarian tumors. Because the ProteoSolve-TD2 buffer also extracts integral membrane proteins, a more complete picture of the tissue proteome can be obtained than with traditional tissue extraction methods.

Figure 1 shows Coomassie-stained 2-D gel images of the proteome of the fresh-frozen metastatic ovarian tumors extracted using (1) 20 mM HEPES buffer or (2) ProteoSolve™-TD2 buffer. Both clarified extracts were diluted 1:8 into ProteoSolve™-IEF (a denaturing IEF buffer) prior to loading the first dimension (pH 3.5-10 IPG strip). Redfin (Ludesi, AB) analysis of the images shows 97% of the 585 spots found were common to both gels in both position and abundance. Only 1 spot was unique to the HEPES extract while 14 were unique to the TD2 extract.

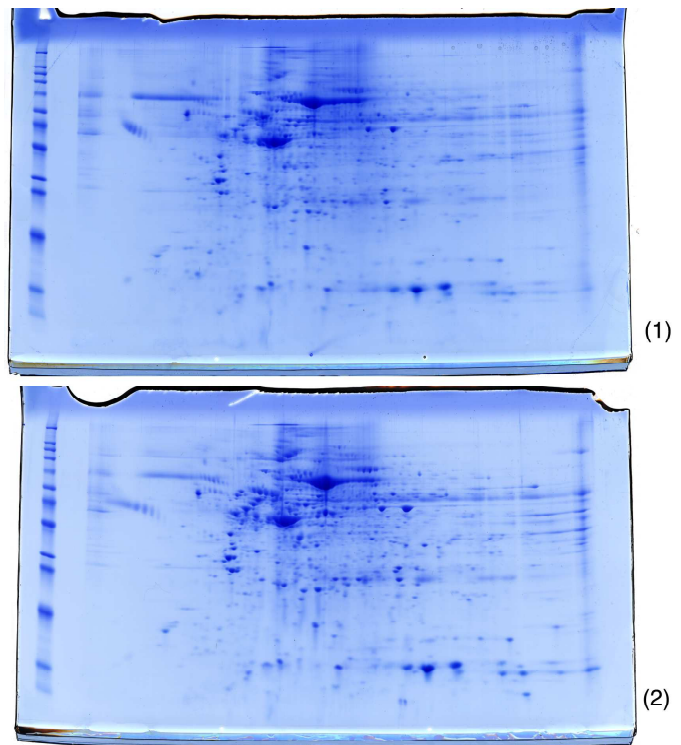
PCT Sample Preparation System (PCT SPS)

The Pressure Cycling Technology Sample Preparation System (PCT SPS) uses rapid cycles of hydrostatic pressure between ambient and ultra high levels to control biomolecular interactions. The PCT SPS can be used to disrupt tissues, cells, and cellular structures to extract proteins and nucleic acids [4-6]. In addition, the PCT SPS can also be used to accelerate enzymatic reactions such as trypsin digestion. The PCT SPS uses a small, semi-automated bench top instrument (Barocycler NEP3229 or the NEP2320) in combination with single-use sample processing containers called FT500 PULSE Tubes (Pressure BioSciences, Inc. South Easton, MA).

PCT Sample Preparation

Pressure cycling technology allows the safe and easy use of very high pressures as a variable in sample preparation. Proteins (including membrane proteins) are solubilized in the ProteoSolve-TD2 buffer system upon return to normal pressure.

High protein recoveries require pre-processing of the solid tissue to obtain high surface area to tissue volume ratios. This was done by cryogenic grinding under liquid N₂ as described in the ProteoSolve-TD2 user manual. The tumor samples used in this study proved intractable to efficient protein extraction with ProteoSolve-TD1 and 20mM HEPES buffer under the same pressure cycling conditions.



Discussion

ProteoSolve-TD2 and PCT provide a simple easy to use alternative to homogenizers for global proteomic analysis of solid tissues. The proteins (including membrane proteins) are extracted in high yield in the ProteoSolve-TD2 buffer system providing a more complete proteomic picture of the tissue. Simply by diluting the clarified ProteoSolve-TD tissue extract into 8 volume equivalents of any suitable denaturing IEF sample buffer, the sample is ready for isoelectric focusing and subsequent 2-D gel analysis via standard methods.

References

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