

Comparative Quantitation of Warburg Effect Proteins in FFPE Tumor and Stroma Tissue From Breast Carcinoma Tumors

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Introduction

- The Warburg effect is the shift of cells from oxidative phosphorylation to glycolysis for the generation of ATP. It is one mechanism by which cancer cells generate energy.
- Recent studies (Pavrides et al. 2009; Cell Cycle 8:23, 3984-4001) have indicated that the Warburg effect may be occurring in fibroblasts and stromal cells rather than the epithelial cells.
- We used laser micro-dissection (LMD) to separate the epithelial and stromal regions from nine breast cancer tissues.
- We used our patented Liquid Tissue-SRM assay technology (Fig.1) to quantify in a relative fashion multiple proteins of the glycolytic pathway from both tumor and stromal compartments.

Methods

- Nine breast cancer tissue blocks were cut on DIRECTOR slides and processed using standard histological procedures.
- Epithelial and stromal tissue were collected using laser micro-dissection.
- The collected tissue samples were then solubilized to tryptic peptides using Liquid Tissue technology (Fig.1).

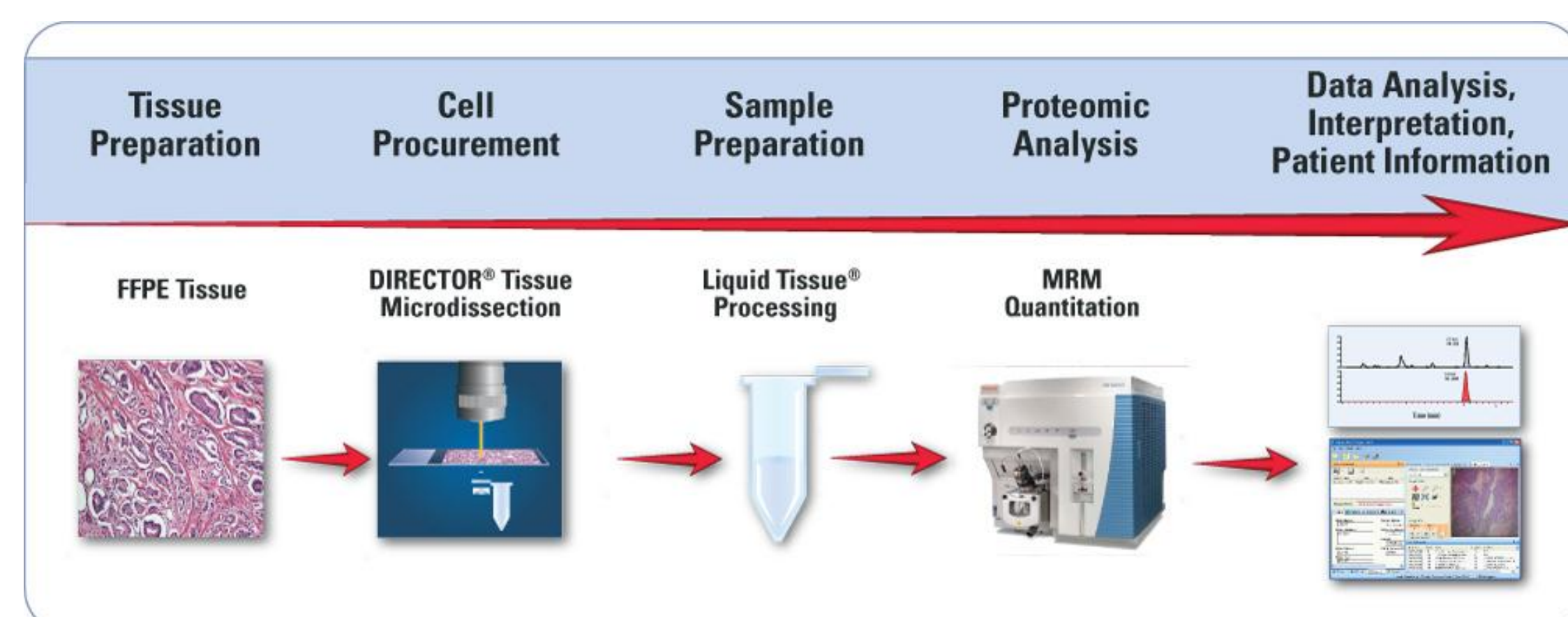


Fig1. Liquid Tissue®-SRM (LT-SRM) workflow for analysis of proteins from FFPE tissue

Selected reaction monitoring (SRM) technology

- Routinely used in pharmaceutical industry for monitoring of drug metabolites.
- One or more peptides from a protein were chosen.
- Relative quantitation (AUC) is based on correct signature of the peptide and compared to an internal control.

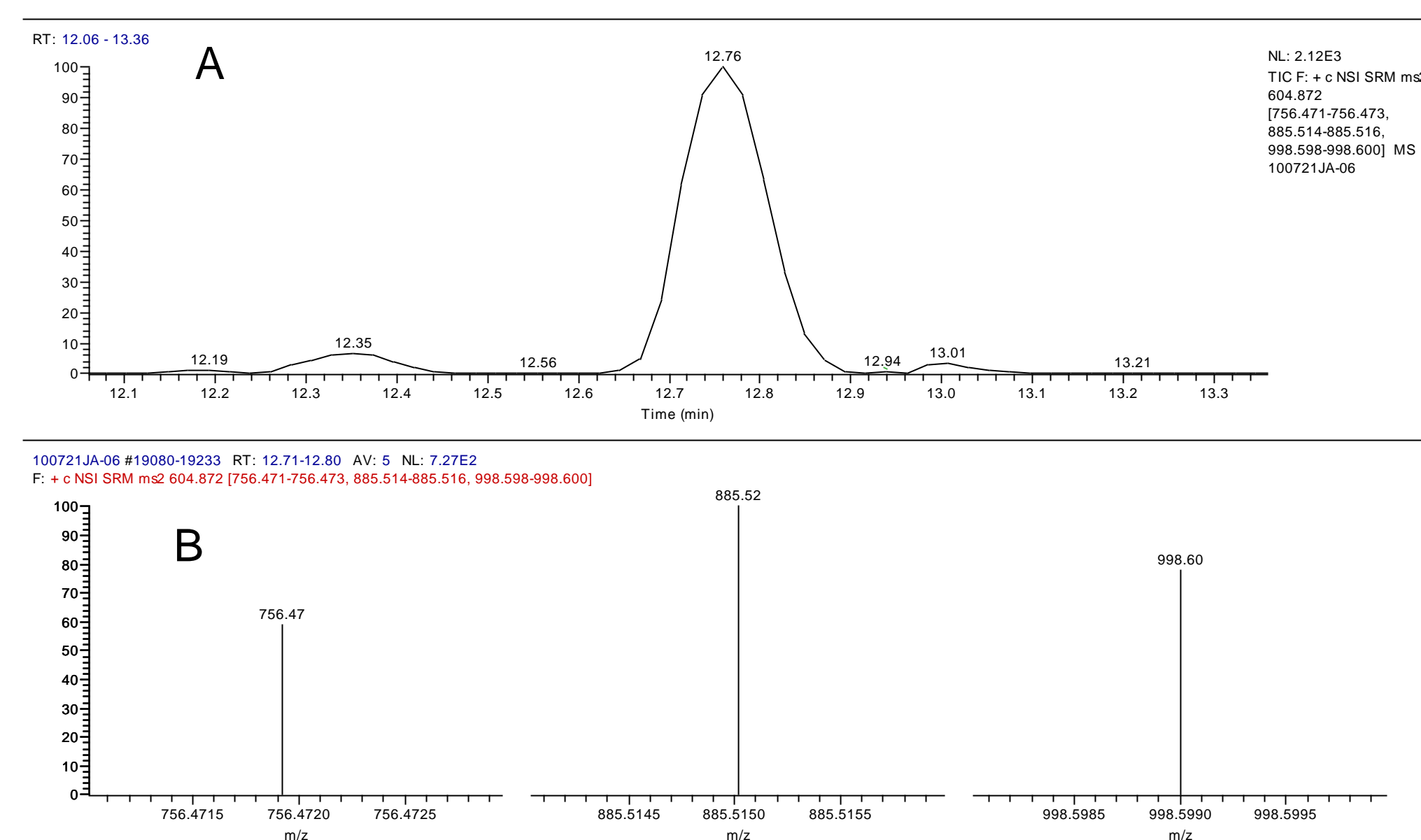


Fig 2. A. Chromatogram trace showing the retention time. B. Spectrum showing the transition ion ratio (signature).

Targets chosen for SRM analysis from FFPE breast cancer tissue

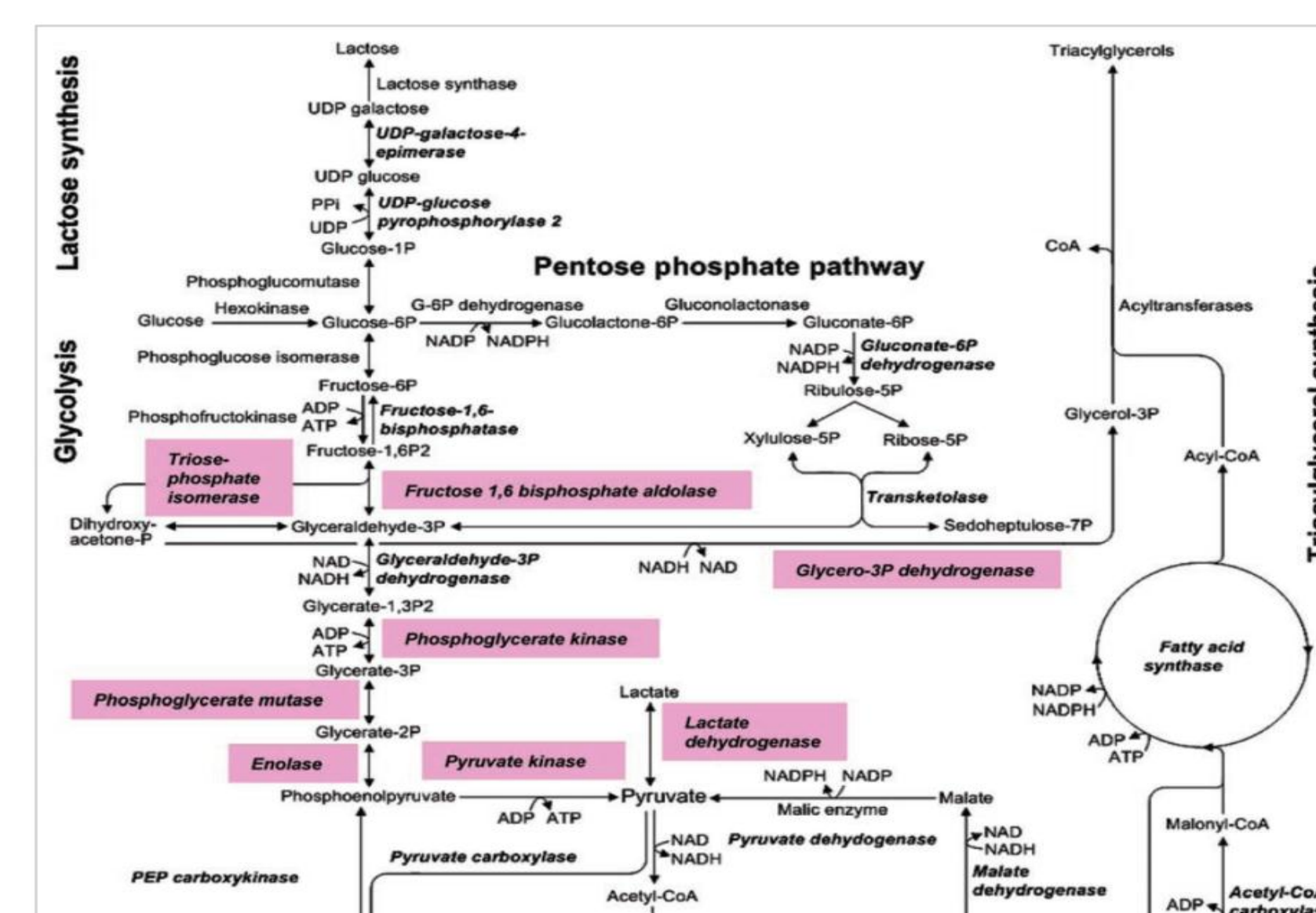


Fig 3. Eight proteins highlighted in pink were chosen as key glycolytic enzymes to be analyzed by SRM (Figure adapted from Pavrides et al. 2009).

Multiplexing capability of SRM assay

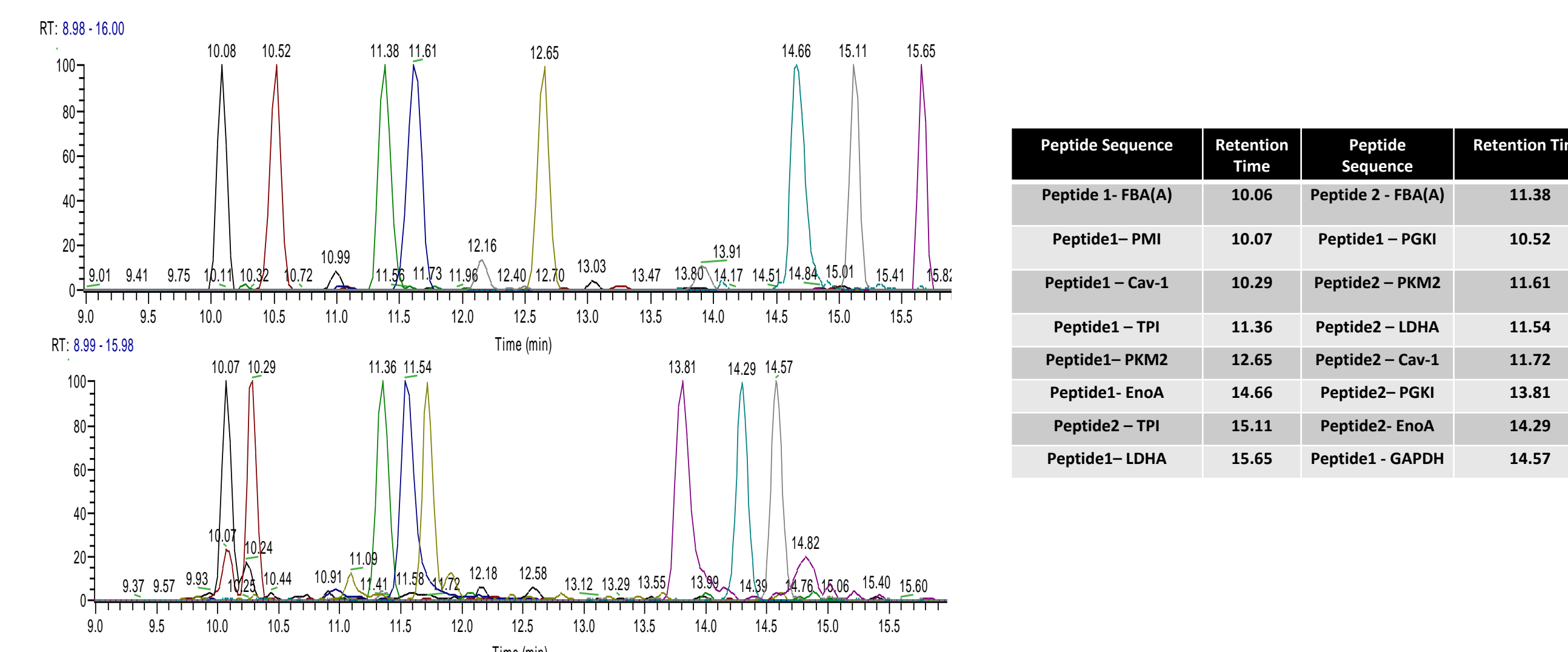


Fig 4. SRM assays are amenable to multiplexing. The figure shows 16 peptides that can be quantified in a single run. In addition, SRM assays are antibody independent, hence less sensitive to preanalytical variations in tissue processing, such as duration of fixation.

Results

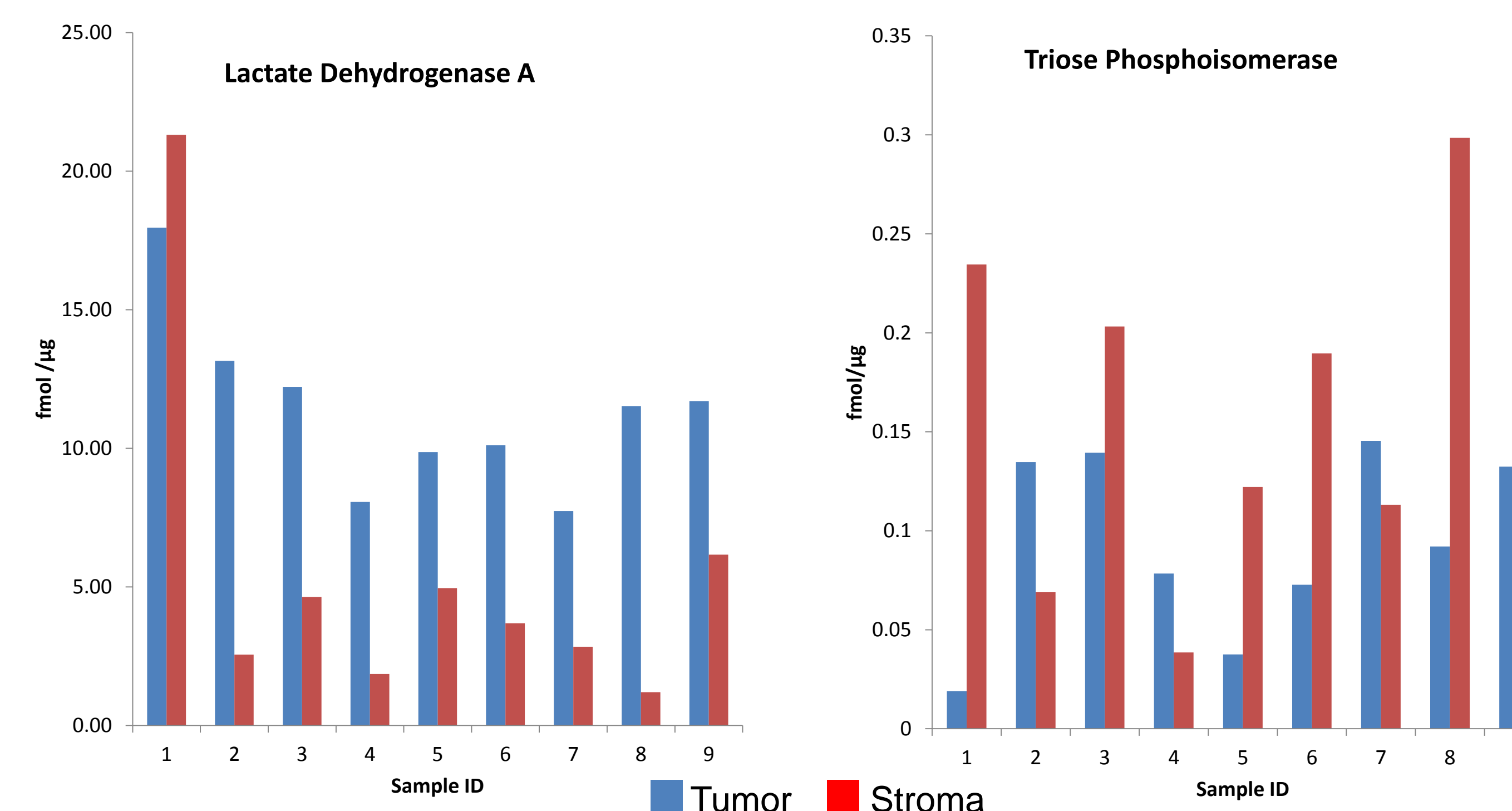


Fig 5. Relative quantitation of A. LDHA B. TPI. This quantitation is normalized based on non-related spiked internal standard. In some instances stromal level of these glycolytic enzymes were above matched tumor epithelial cell expression.

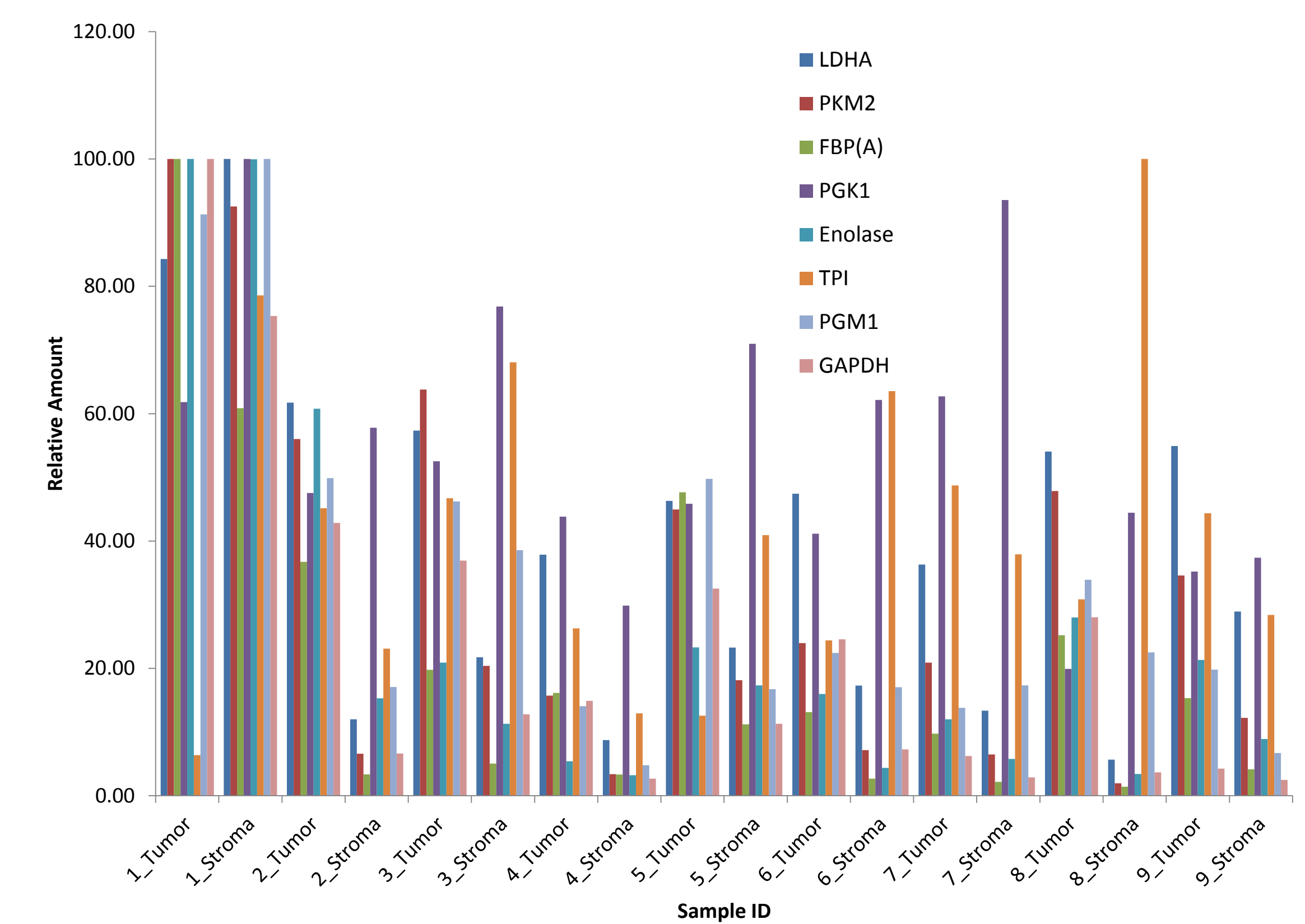


Fig 6. Relative amounts of different glycolytic enzymes in the epithelium and stroma from nine breast cancer samples. Equivalent amounts of protein were analyzed between all samples. Broadly divergent levels of enzyme expression were observed across nine samples.

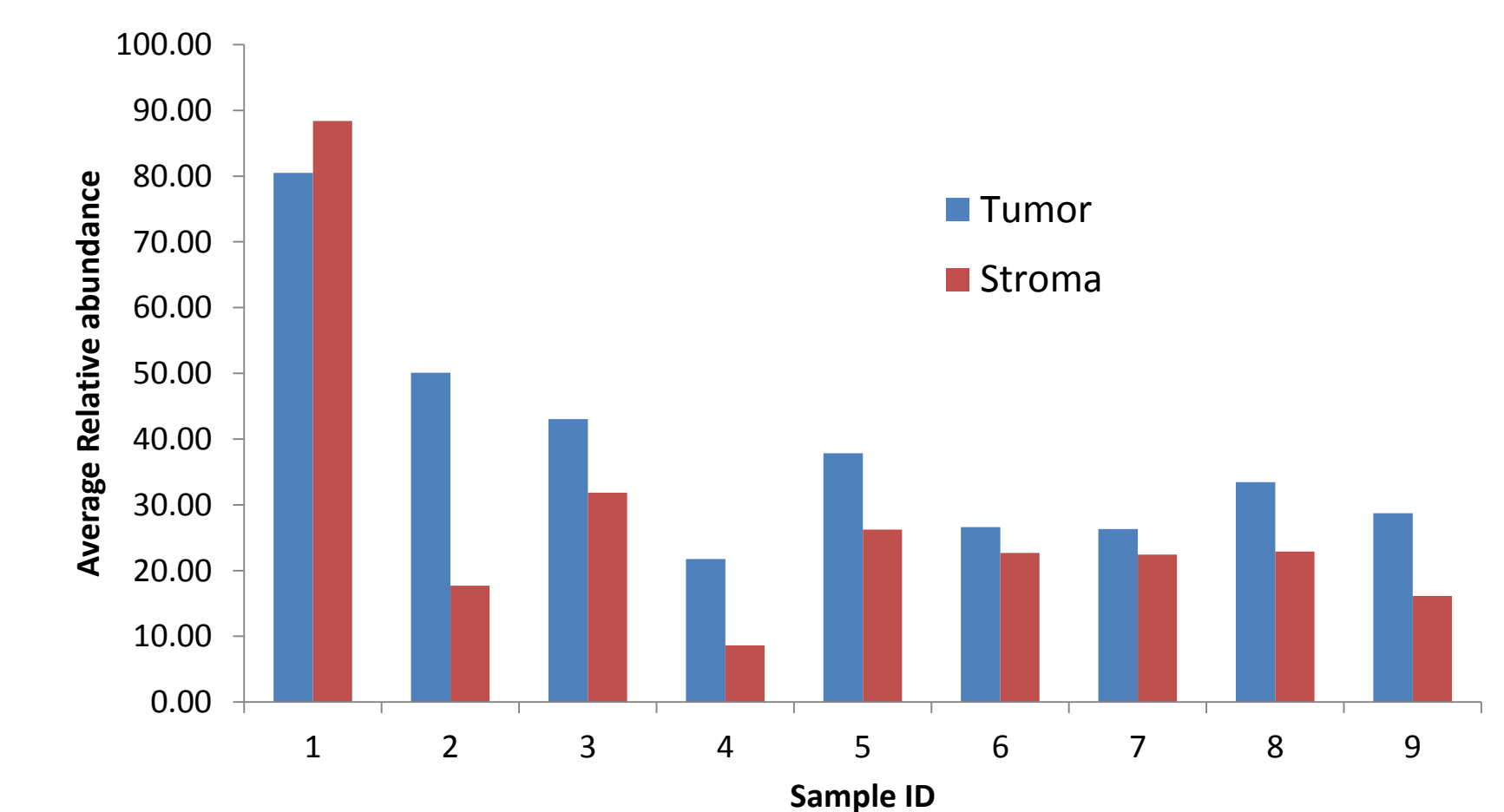


Fig. 7. An average of the eight glycolytic enzymes for nine matched pairs of tumor and stroma shown in Fig. 6.

Conclusion

- Using laser microdissection and Liquid Tissue-SRM assays we can independently characterize protein expression in tumor/ stroma components from FFPE tissue.
- LT-SRM technology can be used to multiplex many analytes from a single injection of 0.5 µg of protein lysate.
- An index of glycolytic enzyme expression can be generated specifically for tumor and/or stromal tissue from FFPE tumor sections.
- Liquid Tissue-SRM analyses of tumor/stromal components of FFPE tissue can be used to address seed vs. soil studies, pharmacodynamic studies, and drug target analysis.