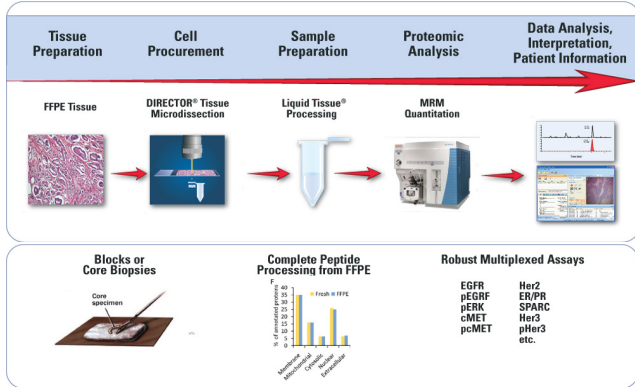


# Quantitative Mass Spectrometric Analysis of EGFR Signaling in FFPE Tissue

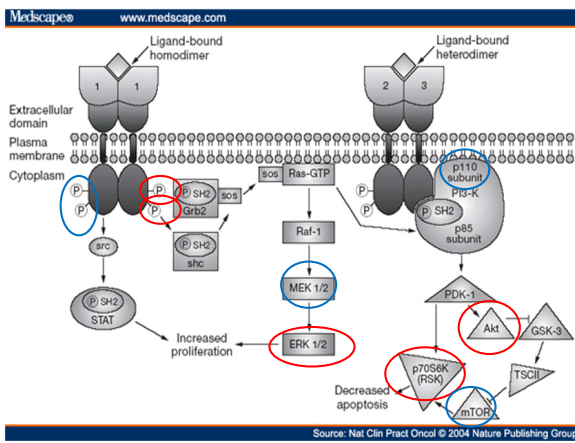
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## EPI's Liquid Tissue® Mass Spec Workflow

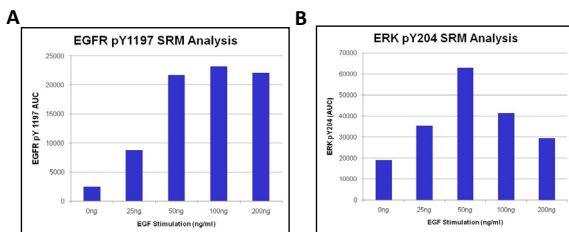


## SRM Analysis of EGFR Signaling



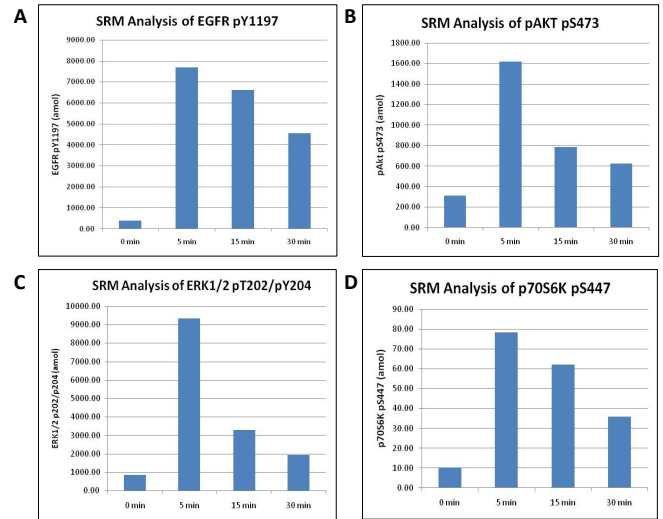
**Figure 1. The EGFR Signaling Pathway.** Measuring protein biomarker levels in patient tissue has promising application to the field of personalized medicine. We have developed a mass spectrometry based tissue proteomics platform capable of measuring protein expression in standard formalin-fixed paraffin-embedded (FFPE) tissue using Selected Reaction Monitoring (SRM) methodology. Targets circled in red have quantitative assays available. Assays for targets circled in blue are currently under development.

## Dose Response Analysis of EGF Signaling in Formalin Fixed A431 Cells



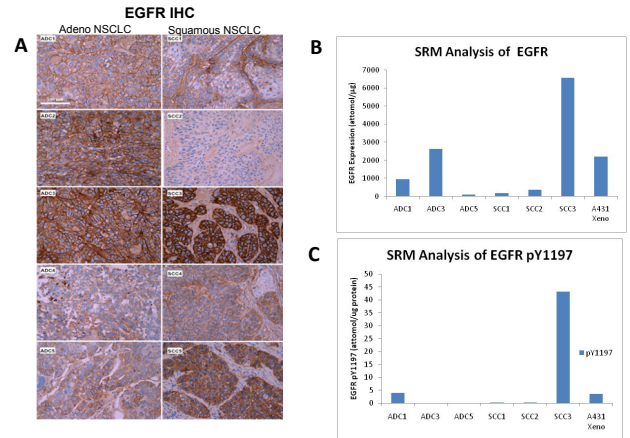
**Figure 2. Dose response of EGF signaling in formalin fixed A431 cells.** Cells were incubated for 5 minutes with increasing concentrations of EGF after which cells were washed, fixed with formalin and subjected to Liquid Tissue processing. 100 µg of cellular protein was phosphoenriched with TiO<sub>2</sub>. SRM analysis of 1/3 of bound material was performed to quantitate EGFR pY1197 (A) and ERK pY204 (B).

## Time Course Analysis of EGF Signaling in Formalin Fixed A431 Cells



**Figure 3. Time Course Analysis of EGF Signaling Formalin Fixed Cells.** Cells were incubated for increasing times with 50 ng/ml EGF after which cells were washed, fixed with formalin and subjected to Liquid Tissue processing. 100 µg of cellular protein was phosphoenriched with TiO<sub>2</sub>. SRM analysis of 1/3 of bound material was performed to quantitate EGFR pY1197 (A), Akt pS473 (B), ERK pY204 (C) and p70S6K pS447 (D).

## Quantitation of EGFR pY1197 in FFPE Human NSCLC Tumor Explant Sections



**Figure 4. Quantitation of EGFR pY1197 from Formalin-Fixed Human Tumor Explants.**

**A.** Immunohistochemical characterization of EGFR in ten NSCLC explants, five adenocarcinoma (ADC1-5) and five squamous carcinoma (SCC1-5). **B.** Quantitation of EGFR pY1197 levels from selected adeno and squamous carcinoma tumors in A. Approximately 50 µg of FFPE tumor protein was used for phosphoenrichment (10 µg for SCC3), and 1/3 of the bound material was subjected to mass spectrometry.

## Summary and Conclusions

The phosphorylation state of multiple components of the EGF receptor signaling pathway were quantitated in formalin fixed tissue sections using a combination of laser microdissection, Liquid Tissue processing, multiple reaction monitoring mass spectrometry. The results provide proof of concept for a robust approach to monitor the EGFR signaling pathway and its activation through phosphorylation.

This new analytical approach offers superior specificity, dynamic range and quantification over traditional antibody-based methods in standard FFPE biopsies of tumors from patients.