

# Efficient immuno-capture based sample clean-up and LC-MS/MS analysis of the early stage small cell lung cancer biomarker ProGRP

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A selective and efficient sample clean-up procedure for ProGRP, an early stage biomarker of small cell lung cancer (SCLC), is described. The method is based on determination of the signature peptide NLLGLIEAK by LC-MS/MS after immuno-capture and tryptic digestion of ProGRP.

The procedure is described in Figure 1. First, ProGRP was captured from serum in 96-wells microtiter plates coated with monoclonal antibodies specific to ProGRP. After immuno-capture and a thorough rinse, trypsin was added for *in-well* digestion. Subsequently the signature peptide was enriched by solid-phase extraction and determined by LC-MS/MS. Different steps in the procedure have been optimised such as dilution of sample, tryptic digestion, and SPE enrichment conditions. A single quadropole MS was used for optimisation of the method. The method was then validated using a triple quadropole MS to improve sensitivity. The brief validation showed good repeatability (RSD: 11.9-17.5%), accuracy (3.0-6.6%) and linearity ( $r^2=0.995$ ) in the tested range (0.5-50 ng/mL). Limits of detection and quantification were in the pg/mL-level enabling determination of clinically relevant concentrations. The method was applied to two patient samples and showed good agreement with an established immunological reference method.

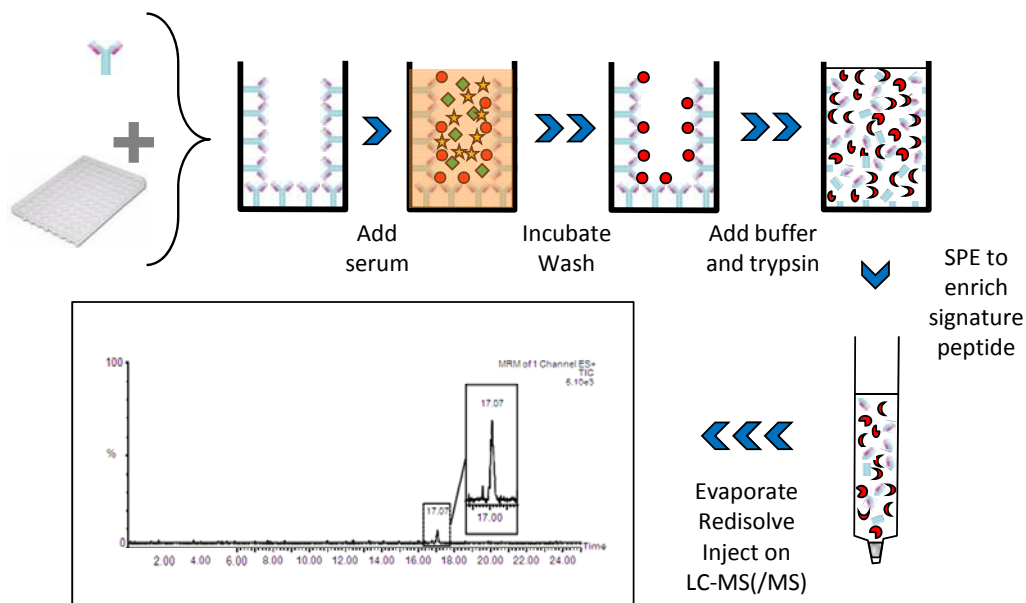


Figure 1. Overview of the complete sample preparation and analysis procedure