PRECISION MEETS DEPTH: PROTEOMIC SOLUTIONS FOR HARD-TO-MEASURE TARGETS

SISCAPA and Biognosys: A Unified Workflow for Accurate Quantitation of Challenging Proteins

SISCAPA (Stable Isotope Standards and Capture by Anti-Peptide Antibodies) is a powerful targeted proteomics technology that enables ultra-sensitive and precise quantification of low-abundance proteins in complex biological matrices such as plasma, dried blood spots, cerebrospinal fluid, serum, and tissue lysates.

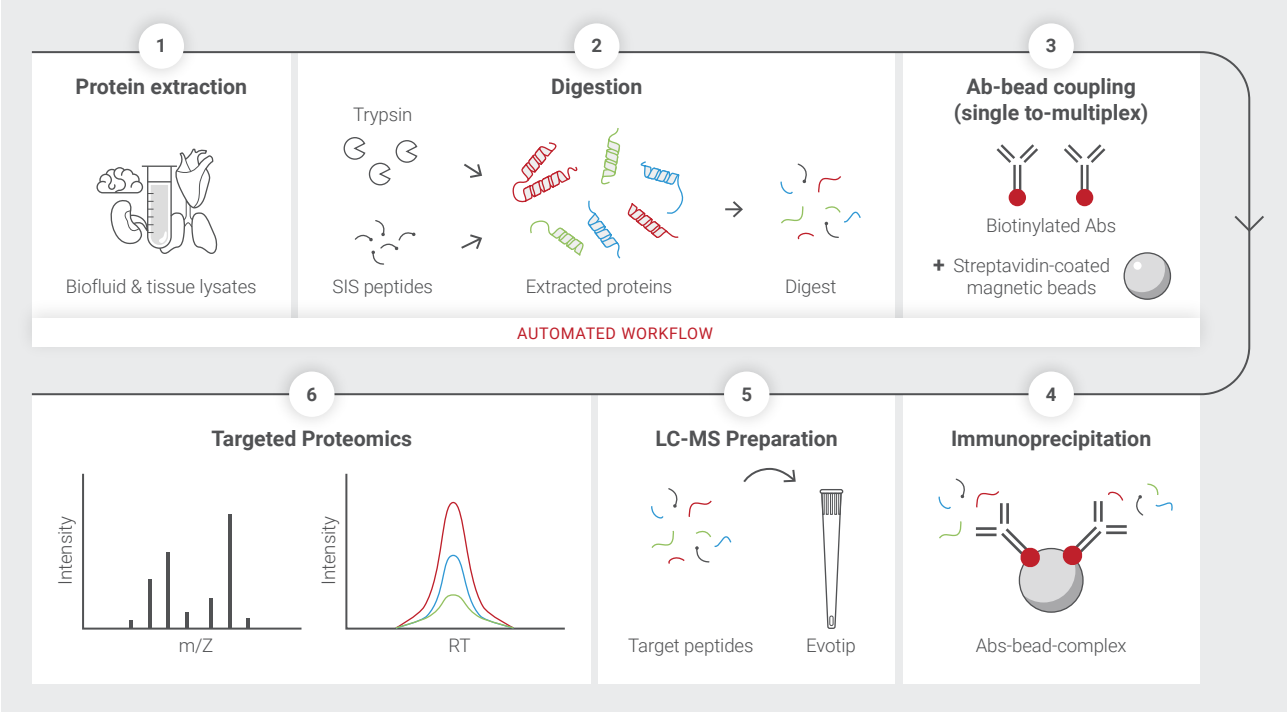
The workflow begins with addition of stable isotope labelled internal standard peptides (SIS) and enzymatic digestion of sample proteins to enable absolute quantification by mass spectrometry. Then, high affinity, highly specific SISCAPA binders (anti-peptide antibodies) are used to isolate the target peptides from background matrix noise. By enriching only the analytes of interest, SISCAPA dramatically improves signal-to-noise ratios and enables accurate measurement of proteins that are otherwise undetectable by traditional methods.

SISCAPA offering is ideally suited for bioanalytical projects requiring high sensitivity and specificity that cannot be achieved with conventional technologies. The method excels in quantifying protein degradation, isoforms, and single-point mutations—critical for applications such as gene therapy and target engagement studies—where immunoassays often fall short due to shared or lost epitopes. It is particularly effective for analyzing transmembrane proteins, which are difficult to solubilize and unstable outside the cellular environment, presenting challenges for traditional immunoassays.

When integrated with Biognosys' high-resolution MS platform, this workflow delivers an end-to-end solution for accurate quantification of proteins with unmatched depth and precision. Coupled with advanced data processing and GCP-compliant options, Biognosys enables streamlined support from discovery to validation and clinical implementation.

This partnership provides a unique and robust platform for researchers and pharma partners aiming to accelerate translational proteomics and confidently quantify the proteins that matter most.

Figure 1. **Biognosys' SISCAPA assay workflow overview**



Case Study

PRECISION MEASUREMENT OF VOLTAGE-GATED SODIUM CHANNELS NAV 1.7 AND NAV 1.8

A SISCAPA Case Study for Low-Abundance, Transmembrane Ion Channel Proteins from Dorsal Root Ganglions (DRG).

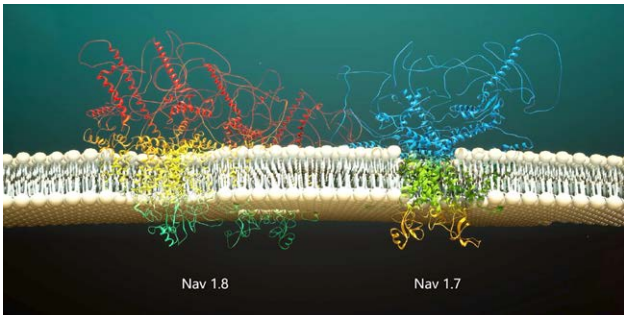
To demonstrate the ability of the SISCAPA workflow to quantify low-abundance, membrane-associated ion channel proteins, we developed and validated targeted assays for Nav 1.7 and Nav 1.8, insoluble membrane proteins involved in pain sensation that are not accessible by conventional immunoassays due to complexity of their interactions with other proteins and lipids at the cell membrane.

Proteotypic peptide targets were selected for both human and rodent isoforms. Custom anti-peptide antibodies were generated and rigorously screened for affinity and specificity. The study included both recombinant protein-spiked and blank authentic matrices from relevant preclinical models. Following the tryptic digestion, SISCAPA immuno-enrichment was performed, and peptides were quantified via targeted MS using SIS internal standards. Linearity, sensitivity, accuracy & precision, and matrix effects were evaluated across multiple sample types, confirming the robustness of the assay for use in drug development and mechanistic studies targeting the pain pathway.

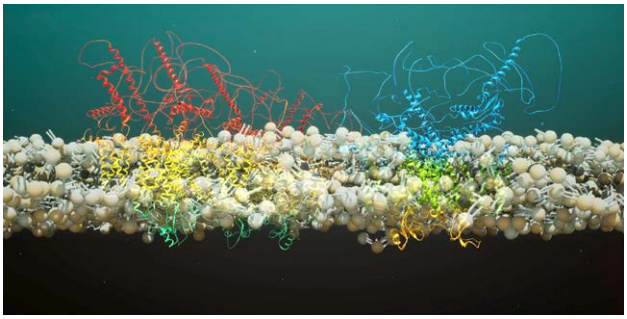
The SISCAPA assays for Nav 1.7 and Nav 1.8 demonstrated excellent sensitivity, achieving lower limits of quantification in the low femtomol on column range, enabling detection of these challenging ion channels in dorsal root ganglion (DRG) samples. Both assays exhibited excellent linearity across a broad dynamic range (over three orders of magnitude) with coefficients of determination (R^2) consistently above 0.99. Precision and reproducibility were confirmed with intra- and inter-assay coefficients of variation below 15%.

As shown in this example, SISCAPA assays enable the quantification of low-abundant proteins that are undetectable by traditional ligand binding assays (LBAs) or direct LC-MS analysis without enrichment. By employing targeted immuno-enrichment, SISCAPA dramatically enhances sensitivity and specificity, overcoming the limitations posed by low abundance and complex biological matrices. This makes it possible to reliably measure proteins that were previously considered inaccessible, providing researchers with powerful tools to unlock new biomarker insights and advance challenging projects.

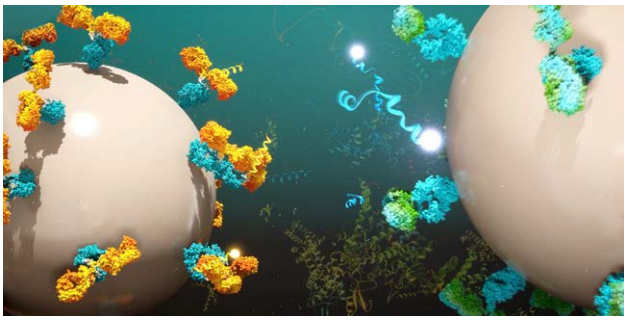
See full results:
https://siscapa.com/wp-content/uploads/2023/12/Poster-NAV1-7_SISCAPA.pdf



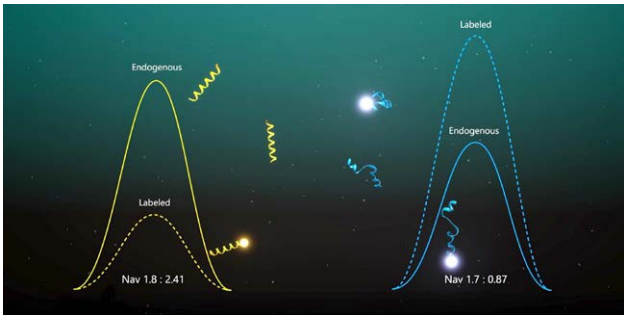
Membrane proteins such as Nav 1.8 and Nav 1.7 sodium channels are challenging to measure.



In SISCAPA, the cell membrane is disrupted and the proteins digested with trypsin.



The target peptides are captured by the antibodies.



Each target peptide is measured against its labeled internal standard by targeted mass spectrometry.

SISCAPA 'OFF-THE-SHELF' MENU

While SISCAPA Assay Technologies has a long track record of developing custom assays, it also offers an expanding menu of ready-to-use assays for the quantification of clinically and biologically relevant proteins, optimized for high-throughput and reproducible performance. The commercial menu includes assays for biomarkers, drug targets, and protein drugs across key therapeutic areas such as oncology, cardiology, inflammation, infectious disease, and metabolic disorders. Each assay is developed using rigorously characterized anti-peptide antibodies and stable isotope-labeled standards, ensuring high sensitivity, specificity, and linear quantification over a broad dynamic range.

These assays are fully compatible with automated workflows and standard LC-MS platforms, making them ideal for translational research, clinical studies, and regulated environments. Custom assay development services are also available for novel targets or low-abundance, complex, hard-to-measure protein targets not covered by the current menu.

Individual Tests

Clinical Specialty Tests

- Adiponectin
- Anti Mullerian Hormone
- Apolipoprotein C-III
- Apolipoprotein E
- CGRP
- Complement C2
- Complement C3
- Complement C9
- Hepcidin
- IGF-1
- Lipoprotein (a)
- Lp-PLA2
- LPS binding protein
- Mannose Binding Lectin
- Myeloperoxidase
- Osteopontin
- Protein C Inhibitor
- Serum amyloid A1
- SHBG
- sCD40L
- Mesothelin
- Thyroglobulin
- TIMP-1
- Vitamin D Binding Protein
- von Willebrand Factor

General Clinical Assays

- Alpha-1-Antitrypsin
- Alpha-2-Antiplasmin
- Alpha-2-Macroglobulin
- Alpha-fetoprotein
- Albumin
- Apolipoprotein A-I
- Apolipoprotein B-100
- Antithrombin III
- CA-125 (MUC16)
- CA 15-3 (MUC1
- Carcinoembryonic antigen
- Ceruloplasmin
- C-reactive protein
- EGFR
- Fibrinogen
- Haptoglobin
- HER-2/NEU protein
- Hemopexin
- Immunoglobulin G3
- Immunoglobulin G1
- Immunoglobulin A
- Immunoglobulin M
- Lipoprotein (a)
- Alpha-1 antichymotrypsin
- Prostate specific antigen
- Soluble transferrin receptor

Research Biomarkers

- CD44
- CETP
- Complement factor H-related Protein 2
- Fibronectin
- Gelsolin
- Ig Lambda light chain
- LRRK2 - FSC
- LRRK2 - HSN
- LRRK2 - MGK
- Lubricin
- Melanotransferrin
- Synuclein alpha

Multiplex Test Panels

Inflammation

- Alpha-1-acid glycoprotein
- C-reactive protein
- Complement C3
- Fibrinogen gamma chain
- Haptoglobin
- Immunoglobulin G total
- Immunoglobulin M
- L-plastin
- LPS binding protein
- Mannose Binding Lectin
- Myeloperoxidase
- Serum amyloid A1

Cardiovascular

- Apolipoprotein A-I
- Apolipoprotein B-100
- Apolipoprotein C-III
- Apolipoprotein E
- C-reactive protein
- Cystatin-C
- Fibrinogen gamma chain
- HbA1c and HbA
- Lp-PLA2
- Lipoprotein (a)
- Myeloperoxidase
- sFlt-1
- TIMP-1
- von Willebrand Factor

Iron Metabolism

- Haptoglobin
- Hemoglobin
- Hemopexin
- Ferritin light chain
- Hepcidin
- Soluble transferrin receptor
- Transferrin

Oncology

- Alpha-fetoprotein
- CRP
- CA 15-3 (MUC1)
- CA-125 (MUC16)
- CEA
- EGFR
- HER-2/NEU protein
- Osteopontin
- Prostate specific antigen
- Mesothelin
- Thyroglobulin

Wellness

- Adiponectin
- Albumin
- Alpha-1-acid glycoprotein
- Apolipoprotein A-I
- Apolipoprotein B-100
- Apolipoprotein C-III
- Apolipoprotein E
- C-reactive protein
- Cystatin-C
- Fibrinogen gamma chain
- Ferritin
- HbA1c and HbA
- IGF-1
- Immunoglobulin G
- Immunoglobulin M
- L-plastin
- Myeloperoxidase
- Serum amyloid A1
- Vitamin D Binding Protein

KEY TAKEAWAYS

Enhanced sensitivity with targeted enrichment
SISCAPA technology selectively enriches target peptides from complex biological samples, significantly boosting signal-to-noise ratios. When combined with SIS standards and targeted mass spectrometry, it delivers highly reproducible and absolute quantification — even for low-abundance proteins.

Advantages over traditional ligand binding assays
SISCAPA addresses key limitations of traditional ligand binding assays by enabling robust quantification that is unaffected by matrix interferences, anti-drug antibodies, or epitope variability. Its superior specificity, multiplexing capability, and compatibility with diverse sample types make it an ideal solution for generating reliable and clinically meaningful insights — even in the most challenging biological matrices.

Off-the-shelf assays for key therapeutic areas
SISCAPA provides off-the-shelf assays for biomarkers and drug targets across oncology, cardiology, inflammation, infectious diseases, and metabolic disorders. These assays are optimized for high-throughput workflows, offering sensitive and specific quantification.

Automated, clinical-grade workflows
With automated sample preparation and GCP-compliant processes, the platform is ideally suited for clinical research. It ensures robust performance, regulatory alignment, and reliable data generation.

Comprehensive services via Biognosys CRO
Biognosys offers end-to-end support from its Switzerland and US sites — including custom assay development, ready-to-use assays, and complete data analysis — through its contract research services.

SISCAPA Assay Technologies (SAT) specializes in developing, performing, and supporting definitive immunoaffinity-MS assays for biologics and targets to protein targets, and biomarkers. Founded in 2011 by pioneers in the practical application of proteomics for pharmaceutical and diagnostic applications, SISCAPA has since developed hundreds of assays, including mass spectrometry tests. Find out more at **siscapa.com**

Discover how Biognosys' SISCAPA targeted mass spectrometry can enhance your preclinical and clinical research with precise protein quantification. Contact us to discuss your goals:
biognosys.com/contact

BIOGNOSYS
NEXT GENERATION PROTEOMICS