PRECISION IN EVERY PROTEIN

TECHNICAL NOTE

NeuroKey-3™, A Quantitative Multiplexed IP-MS Assay to Measure Key Low-Level Markers Linked to Neurodegeneration

Introduction

The available treatments for neurodegenerative diseases are restricted, and the effective advancement of innovative therapeutics in the future, as well as the assessment of their effectiveness beyond traditional cognitive tests, will heavily depend on an optimal biomarker strategy for the identification, monitoring, and stratification of these diseases[1]. However, assay development and biomarker validation for current offerings is a costly and laborious process, most of which do not meet the criteria for regulatory submission.

To address these challenges, Inoviv developed NeuroKey-3™, a proprietary assay that utilizes analytical algorithms, workflows, and internal standards, providing high reproducibility, robustness, sensitivity, and a linear dynamic range over six orders of magnitude. This offers absolute quantification at low pg/ml concentration following immunoprecipitation-based enrichment (IP-MS) in a hybrid assay format.

For those involved in the development of neurological drugs requiring a multiplexed IP-MS assay to measure key proteins associated with neurodegeneration in human plasma, NeuroKey-3™ provides a solution to expedite their clinical trial workflows.

NeuroKey-3™ is fully validated following the FDA's Bioanalytical Method Validation guidelines for data submission to regulators as exploratory endpoints.

Definitions

Immunoprecipitation-Mass Spectrometry (IP-MS): Utilizes antibody-enabled enrichment prior to sample digestion and LC-MS/MS analysis to reduce sample complexity. Currently, it is the gold standard for quantification of low-level biomarkers (e.g. neurodegeneration; Amyloid-β isoforms and Phospho-tau-217).

Liquid Chromatography-Mass Spectrometry (LC–MS): Method to seperate compounds in a sample before analysis followed by mass-spectrometry based detection.

NeuroKey-3 Assay & Method

The NeuroKey-3 IP-MS assay was developed and validated for the determination of Neurogranin (NRGN), Interleukin-18 (IL18), and Brain-Derived Neurotrophic Factor (BDNF) concentrations in human plasma **(see table)**. Inoviv's IP-MS based NeuroKey-3™ workflow consists of 3 steps **(Figure 1).**

NeuroKey-3 Developed & Validated Markers:

Biomarker	Disease Process	Biomarker Function
Interleukin-18 (IL-18)	Inflammation	Mediates neuroinflammation in the CNS[2]
Neurogranin (NRGN)	Synaptic Degeneration	Associated with cognitive decline, involved in synaptic plasticity[3,4]
Brain-derived neurotrophic factor (BDNF)	Neurotrophic Factor	Essential in neuronal survival & growth[5]

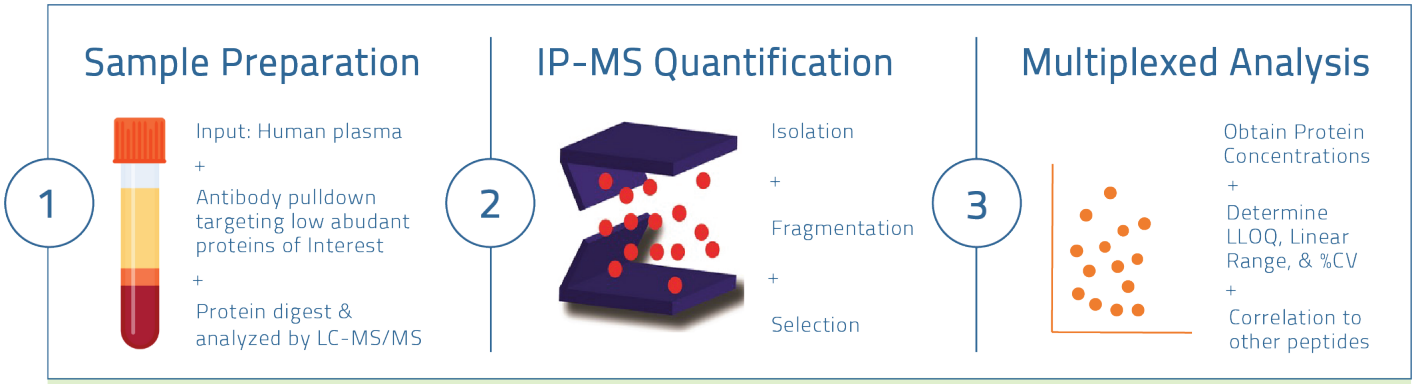


Figure 1. The NeuroKey IP-MS Workflow. (1) Individual monoclonal antibodies were pooled together and incubated with IS-spiked 400 pL of plasma for immunocapture of the protein of interest. The capture procedure was optimized on the KingFisher robotic system (Thermo) in 96W format for a high throughput sample preparation. The captured proteins were digested into peptides and analyzed by LC-MS/MS. **(2)** Waters Premier UPLC coupled to SCIEX 7500. **(3)** A linear regression model was fitted through the calibration line samples to obtain absolute protein concentration. Calibration lines are plotted on logarithmic axes (log-log). Principal component analysis, Spearman's correlations of each peptide to other peptides and other statistical analyses were performed to analyze the generated dataset.

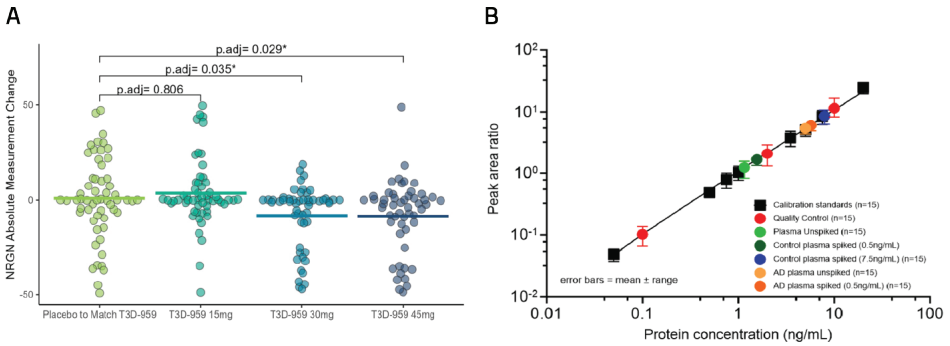
Results

Targeted Proteomics

Individual biomarker plots, based on absolute protein concentrations, demonstrate how the levels of each biomarker change in response to treatment at three different doses **(Figure 2A)**. IP-MS capabilities can quantify low abundance protein biomarkers following treatment. The technology platform can be adapted to provide definitive data for PTMs, lipids, and metabolites.

Analytical Validation

Across the full quantification range, low coefficient of variation demonstrates excellent inter- and intra- day precision. Acceptance criteria for calibration points are <20% CV and 80-120% accuracy (n=3). Lower limit of quantification is determined as the lowest calibration curve point passing both criteria. A standard biomarker report contains statistical analysis, graphical data representations, quantitative values, and analytical performance metrics **(see table)**.



Linear range tested from (ng/mL)	0.05
Linear range tested to (ng/mL)	50
Lower limit of detection (LLOD, pg/mL)	125
Lower limit of quantification (LLOQ, pg/mL)	125
Upper limit of quantification (ULOQ, ng/mL)	50
Mean conc. in control plasma (ng/mL)	2.89
Median accuracy (%) – standards in BSA	99.6
Intra-day precision (%CV) – standards in BSA	8.0
Inter-day precision (%CV) - standards in BSA	11.0
Accuracy (%) – plasma	96.1
Intra-day precision (%CV) – plasma	8.5
Inter-day precision (%CV) - plasma	11.3

Figure 2. (A) Neurogranin (NGRN) levels measured with IP-MS are monitored for three different doses or a placebo. NGRN levels are decreased following treatment at two different doses (30mg, 45mg). **(B)** Calibration lines for NGRN were generated in five replicates each day (intra-day validation) over 3 days (inter-day validation).

Conclusions

- NeuroKey-3™ has been successfully developed & analytically validated with low abudant proteins showing excellent performance metrics
- Biomarker data generated from NeuroKey-3™ are available to use as an exploratory endpoint in clinical trials for new AD treatments seeking regulatory approval

References

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