NULISAqpcr™ AD 5-plex

DATA SHEET

Introduction

Fluid biomarkers are revolutionizing the diagnosis and management of neurodegenerative diseases by enabling earlier diagnosis and disease monitoring. In particular, blood-based biomarkers have emerged as a minimally invasive and scalable alternative to cerebrospinal fluid analysis. The NULISAqpcr AD 5-plex Assay (for Research Use Only (RUO)) integrates biomarkers across the ATNI framework—Amyloid (A), Tau (T), and Neurodegeneration (N) and Inflammation (I), providing precise and simultaneous quantitative measurement of five key biomarkers—BDpTau217, NfL, Aβ42, GFAP, and APOE4 carrier status all from a single, non-invasive blood or plasma sample. Designed for both ease and accuracy, the AD 5-plex assay provides an integrated view of Alzheimer's pathology making it an invaluable tool for clinical research.

Biomarker Details

BD-pTau217 is a brain-derived (BD) phosphorylated tau isoform and a highly specific biomarker of Alzheimer's disease, with levels rising earlier than other p-tau species during the initial stages of Alzheimer's disease pathology. The NULISAqpcr BD-pTau217 assay selectively detects pTau217 derived from the brain in either blood or cerebral spinal fluid (CSF), enabling precise measurement of this tau isoform that reflects the true brain status.

Aβ42 is a key proteolytic fragment of the amyloid precursor protein and robust indicator of underlying brain amyloid pathology. Consistently lower plasma levels are observed in Alzheimer's patients compared to cognitively unimpaired individuals. When combined with complementary markers such as p-tau, Aβ42 provides a robust indicator of underlying brain amyloid pathology.

Neurofilament Light Chain (NfL) is a crucial blood and CSF biomarker for assessing brain health, indicating neuronal damage from brain injury or neurodegenerative diseases. The amount of protein released in CSF following axonal damage correlates with concentration. Elevated levels of NfL serve as a sensitive measure of neural injury, providing researchers with a robust tool to quantify and track the progression of neurodegenerative pathology. This makes it an essential biomarker for characterizing disease mechanisms in studies involving Traumatic Brain Injury (TBI), Amyotrophic Lateral Sclerosis (ALS), Alzheimer's Disease (AD), and other neurological disorders.

Glial fibrillary acid protein (GFAP) is an inflammatory marker of astroglial activation, astrocytosis, and astrocytic injury that shows significantly elevated plasma levels in Alzheimer's disease compared with cognitively unimpaired individuals. Plasma GFAP increases prior to measurable cognitive decline, which correlates with disease severity, and demonstrates stronger associations with amyloid-B pathology than CSF GFAP, including the asymptomatic stages. Integration of GFAP with complementary biomarkers supports its use as a plasma or serum marker for exploring early disease-associated biology, amyloid status stratification, and longitudinal progression trends.

APOE4 is the strongest known genetic risk factor for sporadic, late-onset Alzheimer's disease, with dose-dependent effect, driving disease biology through pathways involving amyloid-B aggregation, tau pathology, synaptic dysfunction, and immune-mediated neuroinflammation. Emerging evidence, including early interventional data in APOE4 homozygotes, indicates that targeting APOE4-specific mechanisms can prevent or modify plaque formation, positioning APOE4 as an active disease biology driver rather than solely a risk stratification marker. APOE4 status is also linked to an increased risk of Amyloid-Related Imaging Abnormalities (ARIA) in patients receiving anti-amyloid monoclonal antibody therapies.

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NULISA™ Technology

NULISA is a sandwich immunoassay in which two target-specific antibodies, conjugated with unique oligonucleotide tags, bind and form a complex with the specific protein in solution. The resulting immunocomplex is purified in sequential capture and release steps to remove background and unbound antibodies. Successful formation of the immunocomplex brings the two oligonucleotide tags into proximity for a ligation reaction and the resulting product read out using quantitative PCR. The NULISAqpcr assay protocol is fully automated on the ARGO HT System, which features an on-board qPCR reader and data output via the ARGO Command Center, requiring less than 30 minutes of total hands-on time.

Assay Performance

Assay performance was determined from 50 independent runs using one manufacturing lot of reagents on four different instrument systems.
Values are calculated using an Absolute Quantitation Master Curve (AQMC) approach with a single calibrator (CAL) sample with known concentrations of target proteins instead of an onboard calibrator series. Parameters for calibration are assigned for each reagent lot, and four replicates of the CAL sample are analyzed to calibrate each run against the target-specific master curves.

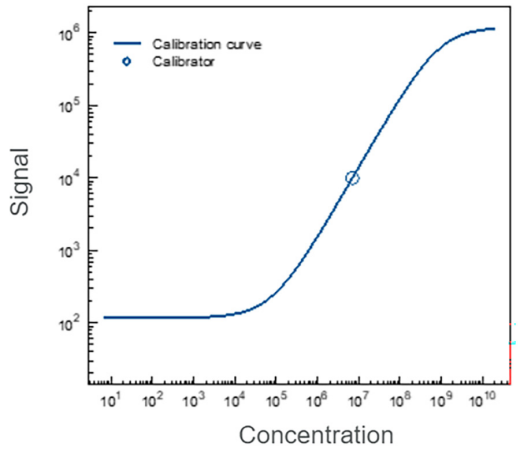

Figure 1: Calibration curve

Table 1: Assay sensitvity and range –analytical and functional

	BD-pTau217		NEFL		GFAP		Aβ42	
	Functional	Analytical	Functional	Analytical	Functional	Analytical	Functional	Analytical
LOD	0.03 pg/mL	0.006 pg/mL	0.04 pg/mL	0.008 pg/mL	0.05 pg/mL	0.010 pg/mL	1.55 pg/mL	0.310 pg/mL
LLoQ	0.14 pg/mL	0.028 pg/mL	0.13 pg/mL	0.026 pg/mL	0.15 pg/mL	0.030 pg/mL	1.93 pg/mL	0.386 pg/mL
ULoQ	35,763 pg/mL	7,153 pg/mL	68,000 pg/mL	13,600 pg/mL	77,844 pg/mL	15,569 pg/mL	989 pg/mL	198 pg/mL
Dynamic Range	5.4 Log^{10}		5.7 Log^{10}		5.7 Log^{10}		2.7 Log^{10}	

The LoD (Limit of Detection) is the lowest concentration of the analyte that can be distinguished from the background signal of the assay and calculated as 2.5 standard deviations above the average of the blanks.

The LLoQ (Lower Limit of Quantification) is the lowest concentration of the analyte that can be reliably quantified by the assay and defined as the lowest concentration measurable by the assay with CV ≤ 25% and 75-125% recovery. The LLoQ was assessed from measurements of serially diluted calibrator material spiked into non-human matrix.

The ULoQ (Upper limit of Quantitation) is the highest concentration of the analyte that can be reliably quantified by the assay and defined as the highest concentration measurable by the assay with CV ≤ 25% CV and 75-125% recovery. The ULoQ was assessed from measurements of serially diluted calibrator material spiked into non-human matrix.

The Dynamic range is defined as the range of concentrations that an assay can reliably quantitate between the LLoQ to the ULoQ.

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Sample Controls

High and low abundance quality control (QC) samples consisting of lyophilized plasma with pre-determined quantities for each target are included in the target kit. These QCs are treated as samples in the run to verify that the generated data consistently meet our stringent accuracy and precision criteria. Lot-specific reference ranges are available in the Certificate of Analysis.

Sample Detectability, Quantifiability & Reference Ranges

Detectability, quantifiability and reference ranges were assessed for each target using 408 plasma samples (68 healthy + 340 diseased). Diseased samples included 32 Alzheimer's Disease, 8 Parkinson's Disease, 93 Cognitively Normal, 163 Mild cognitive impairment, and 44 Dementia samples.

Table 2: Analytical and functional sensitivity and range

Target	Category	Detectability (%)	Quantifiability (%)	Average (pg/mL)	Median (pg/mL)	Min (pg/mL)	Max (pg/mL)
BD-pTau217	Normal	97.10	20.80	0.07	0.05	< LoD	0.55
	Diseased	100	70.30	0.91	0.38	0.01	86.71
	Total	99.50	59.60	0.77	0.23	< LoD	86.71
Aβ42	Normal	100	87.50	18.8	19.8	0.9	40.1
	Diseased	100	99.70	28.99	21.53	1.55	191.7
	Total	100	98.90	28.2	21.48	0.9	191.7
NEFL	Normal	100	98.50	5.21	2.89	0.07	101.96
	Diseased	100	100	13.32	9.17	1.09	270.41
	Total	100	99.80	11.97	8.17	0.07	270.41
GFAP	Normal	100	100	5.97	4.79	0.18	30.69
	Diseased	100	100	24.73	19.37	2.39	261.37
	Total	100	100	21.6	17.16	0.18	261.37

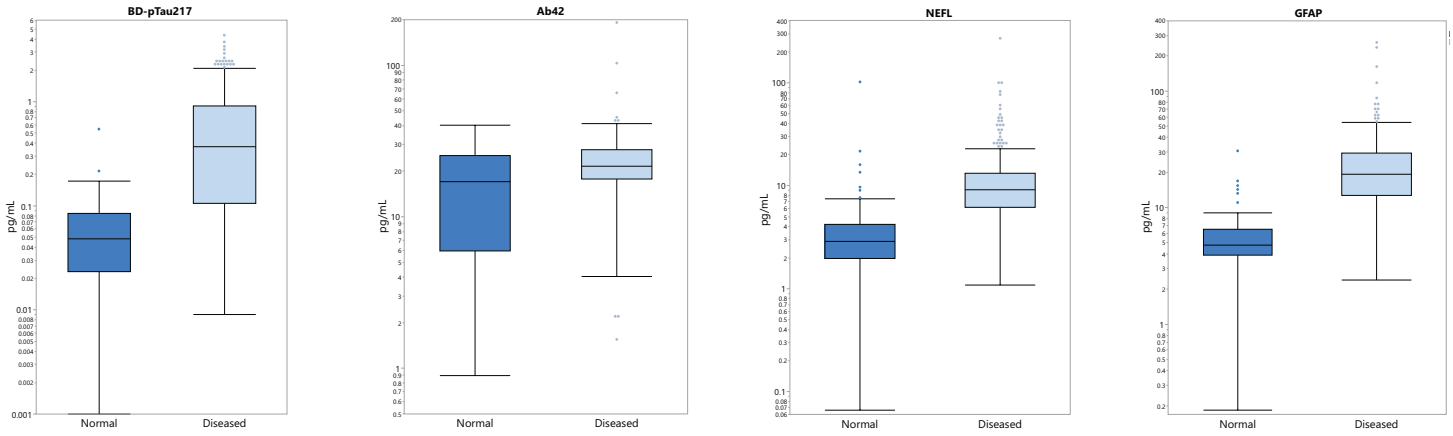

Figure 2: Target concentrations in normal (dark blue) and diseased (light blue) samples

Precision

Intra-assay precision (repeatability) assesses the variability of results obtained when a single sample is tested multiple times within the same assay run. Inter-assay precision (reproducibility) assesses the variability of results obtained when the same sample is tested across different assay runs and/or on different days. Precision was estimated using variance component analysis based on linear mixed-effects models (LMMs), using data from 20 independent assay runs testing a panel of 9 K2EDTA plasma samples with 3 reagent lots across 5 days.

Table 3: Precision and reproducibility

	CV (%)											
	BD-pTau217			Aβ42			NEFL			GFAP		
	Intra-Assay	Inter-Assay	Total	Intra-Assay	Inter-Assay	Total	Intra-Assay	Inter-Assay	Total	Intra-Assay	Inter-Assay	Total
Average	8.9	5.9	15.3	8.1	9.1	13.1	6.0	7.5	13.1	6.2	7.3	12.2
Median	7.2	6.5	13.6	7.9	9.5	12.9	5.9	7.8	12.9	6.4	7.5	12.3
Min	6.0	0.0	11.8	6.5	7.0	11.2	4.3	6.3	11.4	4.8	4.8	10.6
Max	22.1	7.7	24.5	10.0	10.6	15.3	7.6	9.1	14.7	7.5	9.2	14.1

Parallelism

Parallelism confirms that the binding characteristic of the endogenous analyte to the antibodies is the same as for the calibrator. Each individual sample undergoes a serial dilution. Subsequently, the dilution series for each sample is assessed for recovery to ensure a consistent response across all samples.

Parallelism was assessed with 11 EDTA plasma samples serially diluted 2x with sample dilution buffer. At least 80% of the samples tested displayed good parallelism with the recovery in the range of 70%-130% across all dilution levels above the assay LLOQ.

Table 4: Parallelism

	Recovery (%)			
	BD-pTau217	Aβ42	NEFL	GFAP
Average	98%	98%	110%	109%
Median	99%	100%	107%	106%
Min	71%	76%	97%	89%
Max	129%	125%	138%	134%

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Spike Recovery

Spike recovery determines if the concentration–response relationship is similar in the calibration curve/buffer and the samples and is a way to look at matrix effect. A known amount of analyte is spiked into the natural test sample matrix and its response is measured (recovery) in the assay by comparison to an identical amount spiked into the standard diluent.

Spike recovery was determined with 5 EDTA plasma samples which were spiked to three concentrations and compared with spike into sample diluent.

$$\%\ Recovery = \frac{Meas.Conc_{\ Spiked\ Donor\ Sample} - Meas.Conc_{\ Unspiked\ Donor\ Sample}}{Meas.Conc_{\ Spiked\ SD\ buffer}} \times 100$$

Table 5: Spike and recovery

	Targeted Spike Conc. (pg/mL)		Recovery	
Target	Level		Average	Median
BD-pTau217	High	50	107%	105%
	Medium	10		
	Low	1		
Aβ42	High	100	89%	87%
	Medium	20		
	Low	2		
NEFL	High	200	80%	86%
	Medium	40		
	Low	4		
GFAP	High	300	76%	77%
	Medium	60		
	Low	6		

Dilution Linearity

Dilution linearity demonstrates that a sample with an analyte concentration above the ULOQ can be diluted to concentration within the assay dynamic range and give a reliable result. Five (5) normal human EDTA plasma samples were spiked with each of the targets and then serially diluted with sample diluent 2x. Percent recovery was normalized to the dilution adjusted, neat concentration. At least 80% of plasma samples showed good recovery within 70-130%.

Table 6: Dilution linearity recovery

Spiked Concentration (pg/mL)				
Dilution Factor	BD-pTau217	Aβ42	NEFL	GFAP
N/A	50.00	100.00	200.00	300.00
1/2	25.00	50.00	100.00	150.00
1/4	12.50	25.00	50.00	75.00
1/8	6.25	12.50	25.00	37.50
1/16	3.12	6.25	12.50	18.75
1/32	1.56	3.13	6.25	9.38
1/64	0.78	1.56	3.12	4.69
1/128	0.39	0.78	1.56	2.34
Recovery %	BD-pTau217	Aβ42	NEFL	GFAP
Average	93%	118%	113%	105%
Median	94%	111%	97%	103%

Interference

Interference testing was conducted using six (6) K2EDTA plasma samples. The highest concentration of interferents that does not cause significant impact on the assay is provided in the table below.

Table 7: Highest tolerable concentration

Interferent	BD-pTau217	Aβ42	NEFL	GFAP	APOE4
Bilirubin_unconjugated	684 µM	684 µM	684 µM	684 µM	684 µM
Bilirubin_conjugated	475 µM	475 µM	475 µM	475 µM	475 µM
Biotin	3,510 ng/mL	3,510 ng/mL	3,510 ng/mL	3,510 ng/mL	3,510 ng/mL
HAMA	1,200 ng/mL	1,200 ng/mL	1,200 ng/mL	1,200 ng/mL	1,200 ng/mL
Hemoglobin	5 mg/mL	2 mg/mL	5 mg/mL	5 mg/mL	10 mg/mL
Rheumatoid Factor	600 IU/mL	600 IU/mL	600 IU/mL	600 IU/mL	600 IU/mL
Triglycerides	500 mg/dL	250 mg/dL	250 mg/dL	250 mg/dL	1,000 mg/dL

Specificity

To ensure that every NULISAqpcr immunoassay accurately detects only the target molecule that it is designed to detect, the cross reactivity was conducted with related proteins and potential interference from chaperones and potential binding partners for each target.

Table 8: Cross reactivity

Cross-Reactant	Tested Conc. (pM)	Observed Cross Reactivity
pTau181	2	None
pTau205	2	0.8% with BD-pTau217
pTau212	2	2.4% with BD-pTau217
pTau231	2	6.7% with BD-pTau217
Total Tau	2	None
Neorofilament medium chain (NEFM)	200	None
Peripherin (PRPH)	88	None
Vimentin (VIM)	9000	None
Aβ40	70	None

Sample Stability and Handling Instructions

Samples should be stored at 2–8 °C for short-term use and at −20 °C or below for long-term storage to preserve analyte stability. Samples should be aliquoted prior to freezing to avoid repeated freeze-thaw cycles.

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Ordering information

NULISAqpcr Assays			
Product Name	**Plate Format**	**Sample Type**	**Catalog Number**
NULISAqpcr AD 5-plex Assay	96	plasma	800172

Consumables & Buffers			
Product Name	**Qty**		**Catalog Number**
NULISA 10X Wash Buffer	1L		801056

Instrument			
Product Name	**Qty**		**Catalog Number**
ARGO HT System	1		800101

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