NULISAqpcr™ BD-pTau217 Assay

DATA SHEET

Introduction

BD-pTau217 is a brain-derived phosphorylated tau protein isoform that serves as a highly specific biomarker for neurodegenerative disease. Tau proteins are distributed throughout the body, but only a fraction found in blood originates from the brain, with the majority secreted by peripheral nerves. Elevated levels of pTau217 in cerebrospinal fluid are a hallmark of Alzheimer's Disease (AD), while increased blood pTau217 is primarily from peripheral sources and associated with other neurodegenerative diseases. The NULISAqpcr BD-pTau217 assay selectively detects pTau217 derived from the brain in either blood or cerebral spinal fluid, enabling precise measurement of this tau isoform that reflects true brain status. It uses only 20 µL* of biological sample and the analytical performance of the NULISAqpcr BD-pTau217 assay has been rigorously validated and the results are described below.

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 has an on-board qPCR reader and data is analyzed using the ARGO Command Center.

Assay Performance

Assay performance was determined from more than 30 independent runs using one manufacturing lot of reagents on 5 different instrument systems.

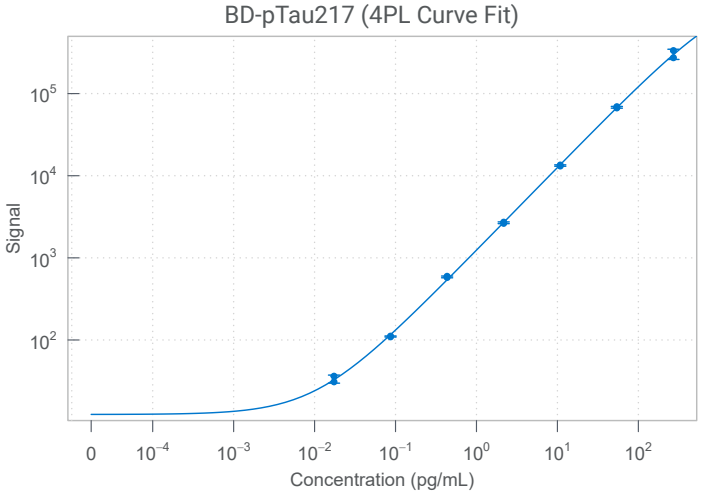

Figure 1: Calibration curve

Table 1: Analytical sensitivity and assay range.

	Analytical	Functional
LOD	0.012 pg/mL	0.06 pg/mL
LLoQ	0.05 pg/mL	0.25 pg/mL
ULoQ	2,931 pg/mL	14,655 pg/mL
Dynamic Range	0.05–2,931 pg/mL	0.25–14,655 pg/mL

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.

**Sample input volumes vary. See product manual for specific requirements.*

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Sample Controls

External controls are High and Low Quality Controls (QCs) included in the target kits, consisting of lyophilized plasma with predetermined BD-pTau217 concentrations.

These QCs are treated as samples in the run to verify that the generated data consistently meet our stringent accuracy and precision criteria.

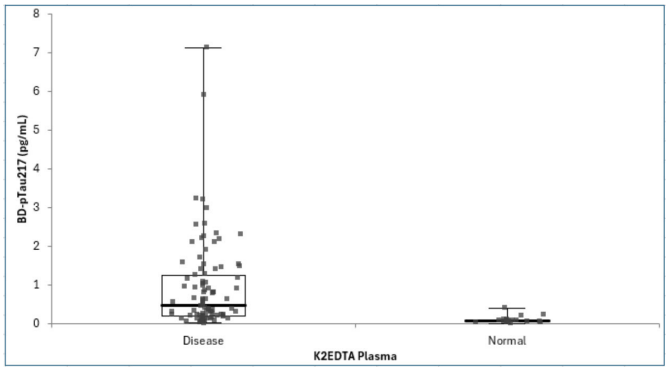
Sample Detectability & Reference Ranges

Sample detectability and reference ranges were established with 20 normal healthy and 100 disease K2EDTA plasma samples.

Table 2: Reference range and detectability

	Plasma (pg/mL), n=120			
	Average	Median	Min	Max
All	0.77	0.306	0.005	7.13
Normals	0.095	0.071	0.005	0.406
Diseased	0.905	0.466	0.017	7.13
Detectability	92.0%			

Figure 2: Signals in normal and diseased samples



Precision

Intra-assay precision or repeatability measures the variation in results obtained when a single sample is assayed multiple times within the same assay run. Inter-assay precision or reproducibility measures the variation in results obtained when the same sample is assayed multiple times in different assay runs or on different days. Precision was determined by ANOVA analysis from a set of three independent runs.

Precision runs included K2EDTA plasma and serum samples.

Table 3: Precision and reproducibility

Sample	Average Conc. (pg/mL)	Total %CV	Inter-assay %CV	Intra-assay %CV
PR-S1	0.39	17.1%	6.8%	15.7%
PR-S2	0.59	11.6%	3.8%	10.9%
PR-S3	1.10	7.3%	0.0%	7.3%
PR-S4	2.18	7.3%	0.0%	7.3%
PR-S5	12.64	19.8%	2.7%	9.4%
PR-S6	112.97	10.4%	5.0%	9.1%

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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 5 EDTA plasma samples and 5 CSF samples serially diluted 2x with sample dilution buffer. 100% of the samples tested displayed good parallelism with the recovery in the range of 70%-130% across all dilution levels above the assay LLOQ.

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 and 5 CSF 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 4: Spike and recovery

		K2EDTA Plasma (n = 5)				CSF (n=5)			
Targeted Spike Conc (pg/mL): Plasma	Targeted Spike Conc (pg/mL): CSF	Avg Recovery	Recovery Range			Avg Recovery	Recovery Range		
1	5	107%	97%	-	118%	102%	90%	-	115%
10	50	106%	85%	-	117%	108%	106%	-	114%
100	680	121%	113%	-	132%	102%	96%	-	112%
	All levels	111%	95%	-	132%	104%	90%	-	115%

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. Normal human EDTA plasma and CSF were spiked with recombinant BD-pTau217 to above ULOQ concentrations and then serially diluted with sample diluent 2x. Percent recovery was normalized to the dilution adjusted, neat concentration. 100% of CSF samples showed good recovery within 70-130%.

Table 5: Dilution linearity recovery.

	Plasma Donor 1	Plasma Donor 2	Plasma Donor 3	Plasma Donor 4	Plasma Donor 5
Starting Concentration (pg/mL)	26.21	24.96	25.73	25.88	25.82
Average Recovery	101%	107%	105%	100%	96%
Min Recovery	98%	102%	95%	95%	85%
Max Recovery	102%	126%	118%	104%	100%

NULISAqpcr™ BD-pTau217 Assay

Specificity

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

Specificity and cross-reactivity were examined by measuring samples spiked with BD-pTau217 and with potential cross-reactants added at three different concentrations. No significant cross reactivity was observed (± 10% difference of test from control) from any of the cross reactants listed in the table at the tested concentrations.

Table 6: Cross reactivity.

Cross-Reactant	Conc. (pg/mL)
Total Tau	128
pTau181	92
pTau205	92
pTau212	92
pTau231	46

Interference

Interference testing was conducted using three concentrations of BD-pTau217 (Low, Medium, and High) in both K2EDTA plasma and CSF matrices. No significant interference was observed from any of the interferents listed in the table at the tested concentrations.

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

Table 7: Analytical specificity and interference.

Substance	K2EDTA Plasma	CSF
Biotin	3510 ng/mL	3510 ng/mL
Bilirubin_conjugated	475 uM	1.0 uM
Bilirubin_unconjugated	684 uM	1.0 uM
Hemoglobin	2 g/L	0.2 g/L
Triglycerides	500 mg/dL	40 mg/dL
HAMA	1200 ng/mL	2.4 ng/mL
Rheumatoid Factor	600 IU/mL	12 IU/mL

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.

NULISAqpcr™ BD-pTau217 Assay

Ordering information

NULISAqpcr Assays			
Product Name	**Plate Format**	**Sample Type**	**Catalog Number**
NULISAqpcr BD-pTau217 Assay	96	plasma/serum/CSF	800470

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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