Amyloid beta mid-domain blood assay

Aβ X-34 PreADx™ Data Sheet

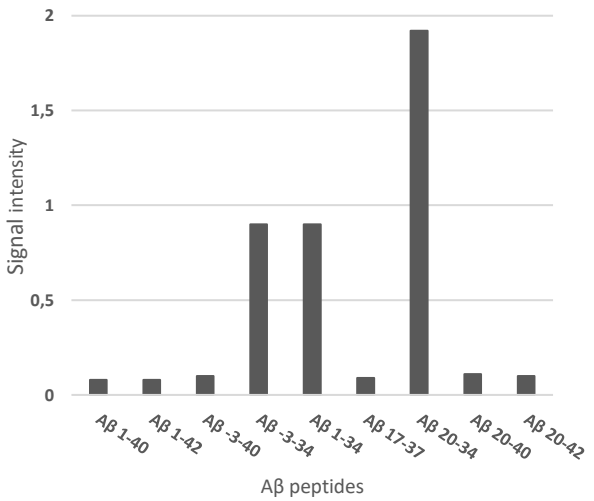
Description: The Aβ X-34 PreADx™ is a digital immunoassay for the quantitative measurements of amyloid beta (Aβ) peptides in monocytes and serum developed for the Quanterix SR-X™ Instrument. This assay is for research use only and not for use in diagnostic procedures.

Deficiency in cerebral Aβ clearance is implicated in the pathogenesis of Alzheimer's disease (AD), and Aβ clearance systems are potential therapeutic targets in AD. Clearance of Aβ is a complex process where phagocytosis and degradation of Aβ are two important components. Innate immune-linked genetic risk factors contribute to AD. Innate immune cells, microglia in the brain and monocytes in the peripheral blood, phagocytose Aβ and contribute to clearance.

Several proteases degrade Aβ both *in vitro* (Rogeberg et al, 2014) and *in vivo* (Torsetnes et al, 2019), at intracellular, cell-membrane and extracellular locations. Activity of these proteases may reflect cellular activation states and may be manipulated for therapeutic purposes. A number of the reported Aβ cleavage sites give rise to Aβ mid-domain peptides (Rogeberg et al, 2015). Two common cleavage sites are located between amino acid residue 19 and 20, and between amino acid residue 34 and 35, generating the Aβ 20-34 peptide.

The PreADx™ assay specifically targets Aβ mid-domain peptides in peripheral human blood samples as a proxy for Aβ degradation. (Fladby US patent: 9,625,474). The sandwich assay utilizes proprietary monoclonal sheep antibodies in combination with labelled recombinant human antibodies.

Sandwich immunoassay specificity: The antibody pair forms a highly selective anti-complex sandwich for Aβ mid-domain, with highest affinity for relatively short peptides with different length (AβX-34). Immunoassay response for a range of synthetic Aβ peptides (1 ng/mL) is depicted below. The y-axis gives the immunoassay signal intensity response for each peptide.



Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted samples along the dynamic range were performed. Concentration and %CV were calculated and used to determine the concentration representing 20 %CV. This concentration was defined as LLoQ.

Limit of Detection (LOD): Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 5 runs each for 1 reagent lot (20 parallels in total).

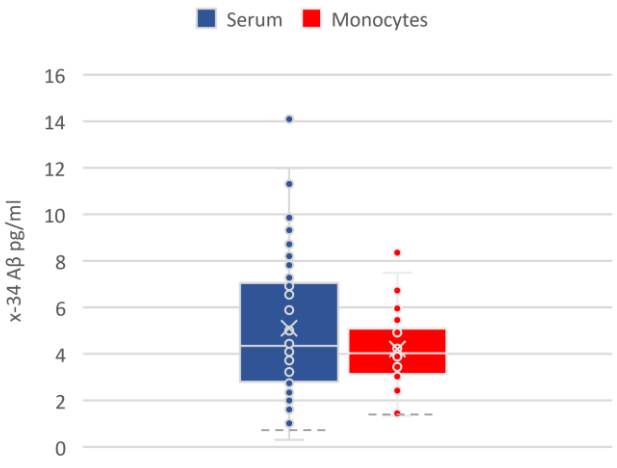
Diluted Sample volume*		100 µL per measurement
Testing		Pre Diagnostics Service lab
	Monocytes	**Serum**
LLOQ	0,74 pg/ml	0,78 pg/ml
LOD	0,43 pg/ml	0,42 pg/ml
Dynamic range	0,43 – 20 pg/ml	0,42 - 135 pg/ml

*20.000 monocytes/ul. Monocyte lysate and Serum diluted 1:2

Sample Reading: Monocyte lysates (n=33) and unmatched serum (n=57) samples were measured. Bars depict median with interquartile range. Dotted lines represent functional LLOQ.

Sample Type	Mean Aβx-34 pg/mL	% Above LOD
Serum	10,2	98%
Monocyte lysate	8,4	100%

Samples diluted 1:2



Diluted sample concentrations shown

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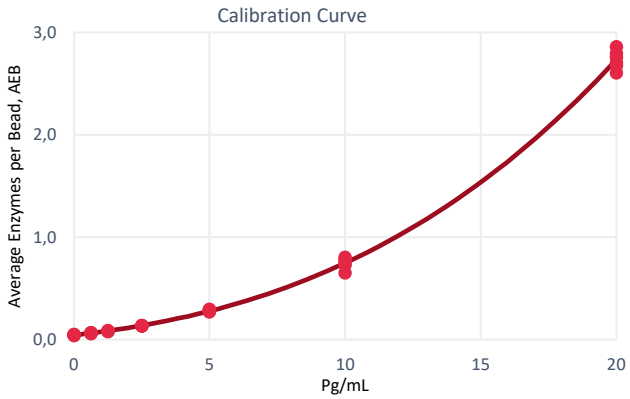
Calibration Curve: Calibrator curve was generated by Assayfit Pro 1.31 using 3rd order polynomial (cubic) curve fit with $1/y^2$ weighting.



Dilution Linearity: A monocyte lysate pool sample was diluted and analyzed in triplicate.

Monocyte lysate pool	Mean concentration measured (pg/mL)	Mean concentration corrected for dilution (pg/mL)	Percent Linearity
Dilution factor 16	14,0	224	100 %
Dilution factor 24	9,7	233	104 %
Dilution factor 32	7,5	241	103 %
Dilution factor 64	4,0	254	105 %
Dilution factor 84	2,8	239	105 %
Dilution factor 96	2,3	217	91 %
Dilution factor 120	2,0	240	111 %

Spike and Recovery: A monocyte lysate pool was spiked with several concentrations within the range of the assay and analyzed in triplicate on the SR-X.

Spiked with Aβ 20-34	Mean fitted conc pg/mL	% Recovery
0 pg/mL	3,14	100%
3 pg/mL	6,27	102%
6 pg/mL	9,01	99%
9 pg/mL	11,93	98%
15 pg/mL	17,83	98%

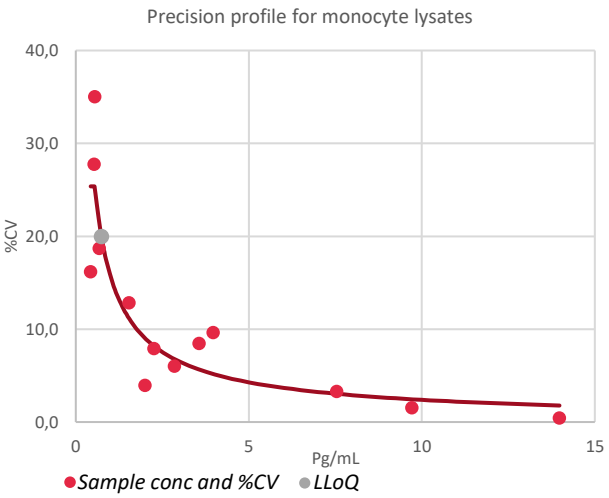
Precision: For within run precision, ten replicates of 2 monocyte lysate pool samples were analyzed.

Monocyte lysate	Mean conc (pg/ml)	Within run %CV
Pool 1	12,71	1,6
Pool 2	2,69	5,0

For between run precision, 4 replicate measurements were made for 5 runs each using 1 reagent lot (20 measurements). Calculated using ANOVA statistics.

Monocyte lysate	Mean conc (pg/ml)	Within run %CV	Between run %CV	Total %CV
Pool 1	12,51	2,7	2,1	3,4
Pool 2	3,17	7,9	0	7,9

The precision profile reflects % CV for mean concentrations of dilutions of monocyte lysates run in triplicate.



Summary: The PreADx™ assay is an accurate and highly sensitive immunoassay for quantification of mid-domain amyloid beta peptides in relevant biological fluids and cells. The assay demonstrates high precision with monocyte lysates and linearity in spiking and dilution experiments.

PreADx™ is compatible with the Quanterix SR-X™ Instrument.

References

Fladby T, Johnsen L, Blennow K. Diagnostic method for Alzheimer's disease. US patent: 9,625,474

Rogeberg M, Furlund CB, Moe MK, Fladby T. Identification of peptide products from enzymatic degradation of amyloid beta. Biochimie. 105, 216-20 (2014)

Rogeberg M, Wettergreen M, Nilsson LN, Fladby T. Identification of amyloid beta mid-domain fragments in human cerebrospinal fluid. Biochimie. 113, 86-92 (2015)

Torsetnes SB, Wettergreen M, Christensen E, Fladby T. Assessing Aβ clearance aided by mass spectrometry. CTAD 2019 Poster P76 (2019).

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