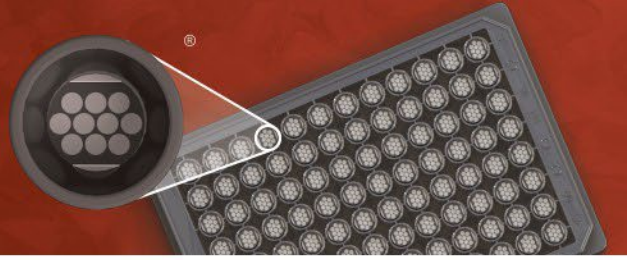
V-PLEX®

Aβ Peptide Panel 2 Kit

Datasheet



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Ordering Information

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Products Covered

Multiplex Kit	V-PLEX	V-PLEX Plus*
V-PLEX® Aβ Peptide Panel 2 (4G8)	K15764D	K15764G
V-PLEX Aβ Peptide Panel 2 (6E10)	K15765D	K15765G
Individual Assay Kits		
Aβ38 Peptide (4G8)	K150AYWD	K150AYWG
Aβ38 Peptide (6E10)	K150AYXD	K150AYXG
Aβ40 Peptide (4G8)	K150AYSD	K150AYSG
Aβ40 Peptide (6E10)	K150AYTD	K150AYTG
Aβ42 Peptide (4G8)	K150AYUD	K150AYUG
Aβ42 Peptide (6E10)	K150AYVD	K150AYVG

*V-PLEX Plus kits contain 3 control reagents in addition to the components in the V-PLEX kit.
Product insert and certificates of analysis are available at https://www.mesoscale.com/en/products_and_services/assay_kits.
For a complete list of products, please visit our website at www.mesoscale.com.

Introduction

The MESO SCALE DISCOVERY® V-PLEX Aβ Peptide Panel 2 Kit measures beta-amyloid (Aβ) peptides derived from amyloid precursor protein (APP) including: Amyloid-beta 1-38 (Aβ38), Amyloid-beta 40 (Aβ40), and Amyloid-beta 42 (Aβ42). The markers of the V-PLEX Aβ Peptide Panel 2 enable the study of Alzheimer's disease (AD) and other neurodegenerative conditions. The V-PLEX Aβ Peptide Panel 2 Kits are available to study these peptides through both the 4G8 and 6E10 epitopes, enabling quantitative study of these important neurodegenerative markers in a variety of studies such as: Aβ aggregation, Amyloid cascade hypothesis, Aβ reduction through small molecule interactions, and other important studies.

Both Aβ Peptide Panels have been validated for human samples. The Aβ Peptide Panel 2 (4G8) is also validated for mouse EDTA plasma.

V-PLEX Characteristics

V-PLEX biomarker products typically provide sensitivity in the pg/mL or better range, enabling detection across a wide range of analyte expression levels.

V-PLEX assays are available as singleplex kits or multiplex panels, which can be customized to create subsets ideal for specific research needs. Grouping the assays into panels by species, analytical compatibility, expression levels, and expected use ensures optimal and consistent performance.

Available reference standards and controls guarantee that every kit provides consistent performance and delivers results that are compatible with international standards. The inclusion of pre-coated V-PLEX plates ensures a simple and reliable workflow that limits sources of variability.

Analytical Validation Data

Analytical validation of the Aβ Peptide Panel 2 Kit was performed using three kit lots. The sensitivity, precision and representative calibration curves were all performed using the Aβ Peptide Panel 2 Kit. All other sections below use data from the Aβ Peptide Panel 1 Kit. Since the only difference between Panel 1 and Panel 2 is the plate and Read Buffer, the Panel 1 performance is expected to be the same for Panel 2. The MSD Methodical Mind Enterprise™ analysis software was used to convert electrochemiluminescence signal to analyte concentrations. Validation data is presented in the following sections.

Calibration Curves

For each plate, a calibration curve was generated by fitting signals from 7 calibrators and a blank to a 4-parameter logistic (4PL) model with $1/Y^2$ weighting. Calibrators were prepared by serial dilution of the recommended top calibrator concentration, with the range designed to ensure the lowest calibrator fell below the expected LLOQ to improve accuracy at low analyte concentrations. Representative calibration curves from one kit lot (minimum 3 replicates across 12 runs) are presented in Figure 1.

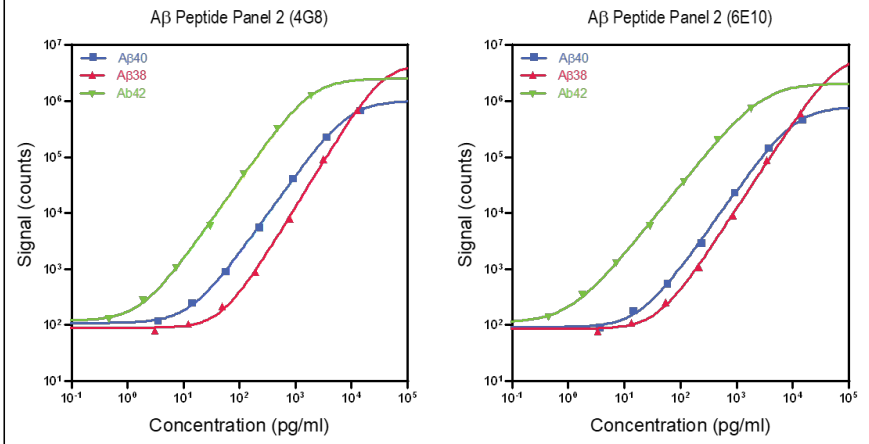


Figure 1. *Representative calibration curves for assays in the V-PLEX Aβ Peptide Panel 2 Kits.*

Sensitivity

The lower limit of detection (LLOD) is a calculated concentration corresponding to the mean signal 2.5 standard deviations above the background (zero calibrator). The LLOD is calculated across multiple plates per lot; the median and range for a representative kit lot are reported in Table 1.

Table 1. *LLOD, LLOQ, and ULOQ for each analyte in the Aβ Peptide Panel 2 Kit*

Analyte	Median LLOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)	Dynamic Range (pg/mL)
Aβ38 (4G8)	29.9	60.0	7,500	29.9 – 7,500
Aβ40 (4G8)	6.67	20.0	6,000	6.67 – 6,000
Aβ42 (4G8)	0.73	2.50	1,270	0.73 – 1,270
Aβ38 (6E10)	27.7	60.0	8,480	27.7 – 8,480
Aβ40 (6E10)	10.1	50.0	7,000	10.1 – 7,000
Aβ42 (6E10)	0.42	3.13	1,270	0.42 – 1,270

The lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) are established by measuring independent samples near the bottom and top of the calibration range, respectively, using the classical method. For the Aβ40 and Aβ42 assays, each limit is set at the concentration where the %CV of back-calculated concentration values is less than 20% and concentration recovery is within 80–120% of the nominal value. The Aβ38 concentration recovery is within 75–125% of the nominal value. Both limits were established across multiple kit lots, are verified for each kit lot, and are reported on the lot-specific Certificate of Analysis available at www.mesoscale.com.

The dynamic range of each assay spans from the LLOD to the ULOQ, typically covering 3–5 log units. The quantitative range, defined as the interval between the LLOQ and ULOQ, typically spans 3–4 log units.

Precision

Independent quality control samples at three different concentrations for each analyte were run on every plate to assess performance of the kit. Precision was based on 22 runs across three kit lots. Each control was tested in triplicate over multiple days (Table 2).

Table 2. Intra-run and inter-run %CVs for each analyte in the Aβ Peptide Panel 2 Kit

Analyte	Control	Average Conc. (pg/mL)	Average Intra-run %CV	Inter-run %CV	Inter-lot %CV
Aβ38 (4G8)	Neurodegeneration Control 1	3,951	8.8%	5.7%	2.7%
	Neurodegeneration Control 2	974	7.2%	3.7%	1.4%
	Neurodegeneration Control 3	216	13.5%	5.4%	0.5%
Aβ40 (4G8)	Neurodegeneration Control 1	7,634	10.4%	5.0%	8.8%
	Neurodegeneration Control 2	2,855	8.4%	4.9%	4.6%
	Neurodegeneration Control 3	429	11.2%	4.6%	0.8%
Aβ42 (4G8)	Neurodegeneration Control 1	1,598	7.6%	4.3%	1.3%
	Neurodegeneration Control 2	551	6.3%	3.5%	2.5%
	Neurodegeneration Control 3	208	9.2%	3.2%	1.4%
Aβ38 (6E10)	Neurodegeneration Control 1	4,130	6.9%	3.9%	4.3%
	Neurodegeneration Control 2	991	6.9%	3.2%	3.6%
	Neurodegeneration Control 3	218	10.3%	4.5%	6.1%
Aβ40 (6E10)	Neurodegeneration Control 1	9,220	10.5%	5.2%	7.5%
	Neurodegeneration Control 2	3,090	10.2%	5.4%	5.2%
	Neurodegeneration Control 3	524	9.6%	5.2%	3.3%
Aβ42 (6E10)	Neurodegeneration Control 1	1,450	5.7%	3.7%	2.7%
	Neurodegeneration Control 2	473	5.6%	2.8%	3.7%
	Neurodegeneration Control 3	187	6.8%	3.2%	3.7%

The intra-run concentration %CV was computed using the triplicates within a plate. The inter-run concentration %CV was computed using all the replicates across the 22 runs. The inter-lot concentration %CV was computed from the average concentration measured within each kit lot. The results show that the average intra-run %CV and inter-lot %CV are all less than 10%. The inter-run %CV is typically less than 10%, well below the acceptance specification of 20%.

Accuracy and precision of controls are measured as part of lot release for each kit lot. Results are reported on the lot-specific Certificate of Analysis available at www.mesoscale.com and meet typical acceptance criteria of %CV less than 20% and recovery between 80–120%.

Sample Ranges

CSF, plasma and diseased samples were commercially sourced. CSF samples were diluted 2-fold prior to testing. Mouse plasma samples were diluted 4-fold prior to testing. Results for each sample type are displayed in Table 3. The difference between normal CSF and Alzheimer's disease (AD) CSF samples are shown graphically in Figure 2. Concentrations are corrected for sample dilution. The median and range are calculated from samples with concentrations at or above the LLOD. The percentage detected is the percentage of samples with concentrations at or above the LLOD.

Table 3. *Human samples tested using the Aβ Peptide Panel 1 Kit*

Analyte	Statistic	Human Patient CSF (N=23)	Pooled Remnant Human CSF (N=12)	Mouse Plasma (N=20)	Normal CSF (N=5)	AD CSF (N=5)
Aβ38 (4G8)	Median (pg/mL)	1,327	670	55.0	NT	NT
	Range (pg/mL)	689-2,910	ND-3,340	ND-217	NT	NT
	% Detected	100	92	15	-	-
Aβ40 (4G8)	Median (pg/mL)	4,162	2,324	207	NT	NT
	Range (pg/mL)	2,122-7,458	58.8-8,347	113-317	NT	NT
	% Detected	100	100	100	-	-
Aβ42 (4G8)	Median (pg/mL)	264	195	25.4	NT	NT
	Range (pg/mL)	37-706	3.96-748	11.7-32.7	NT	NT
	% Detected	100	100	100	-	-
Aβ38 (6E10)	Median (pg/mL)	1,293	745	NT	842	837
	Range (pg/mL)	607-3,202	ND-3,437	NT	761-1,515	418-1,635
	% Detected	100	92	-	100	100
Aβ40 (6E10)	Median (pg/mL)	3,831	2,225	NT	2,715	1,831
	Range (pg/mL)	1,300-7,187	43.7-7,012	NT	2,320-4,827	43.7-4,057
	% Detected	100	100	-	100	100
Aβ42 (6E10)	Median (pg/mL)	187	178	NT	197	56
	Range (pg/mL)	25-607	3.31-691	NT	121-542	3.31-285
	% Detected	100	100	-	100	100

NT = Not Tested
ND = Not Detectable

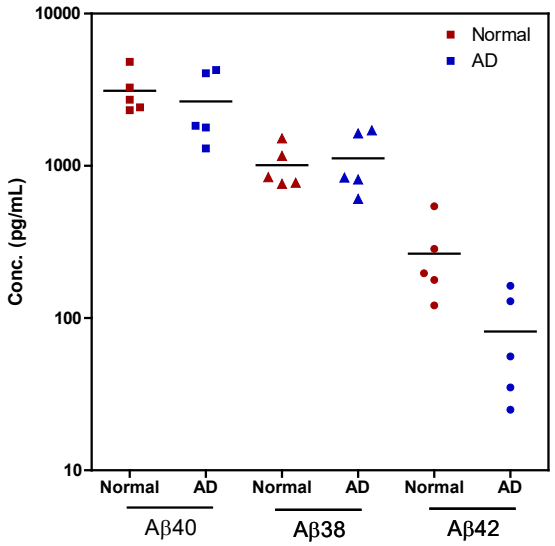


Figure 2. *Normal vs AD CSF samples tested in the V-PLEX Aβ Peptide Panel 1 (6E10) Kit.*

Dilution Linearity

To assess linearity, commercially sourced normal human CSF samples and mouse plasma samples were diluted 2-fold, 4-fold, and 8-fold before testing. Percent recovery at each dilution level was normalized to the dilution-adjusted, 2-fold concentration (human CSF samples) or 4-fold (for mouse plasma samples). The average percent recovery shown below (Table 4) is based on samples within the quantitative range of the assay.

$$\%\,recovery = \frac{measured\ concentration}{expected\ concentration} \times 100$$

Table 4. *Percent recovery at various dilutions in different sample types in the Aβ Peptide Panel 1 Kits.*

Analyte	Fold Dil.	Human CSF (N=10)		Mouse Plasma (N=10)	
		Av.% Rec.	%Rec. Range	Av.% Rec.	%Rec. Range
Aβ38 (4G8)	2	100	-	96	84-105
	4	99	93-103	100	-
	8	94	86-94	101	95-108
	16	NT	NT	105	94-119
Aβ40 (4G8)	2	100	-	93	89-98
	4	110	103-118	100	-
	8	110	105-119	99	97-104
	16	NT	NT	98	94-104
Aβ42 (4G8)	2	100	-	93	89-100
	4	103	96-108	100	-
	8	98	87-103	104	103-109
	16	NT	NT	108	102-115
Aβ38 (6E10)	2	100	-	NT	NT
	4	102	96-102	NT	NT
	8	99	92-107	NT	NT
Aβ40 (6E10)	2	100	-	NT	NT
	4	108	101-114	NT	NT
	8	109	101-125	NT	NT
Aβ42 (6E10)	2	100	-	NT	NT
	4	105	103-113	NT	NT
	8	101	95-111	NT	NT

Fold Dil. = Fold Dilution, Av.% Rec. = Average % Recovery, %Rec. Range = % Recovery Range, NT = Not Tested

Spike Recovery

Spike recovery measurements of different sample types were evaluated throughout the quantitative range of the assays (Table 5). Tested matrices were commercially sourced normal human CSF samples and mouse EDTA plasma samples. Samples were spiked with calibrators and then diluted 2-fold (human CSF samples) or 4-fold (mouse plasma samples). The average percent recovery for each sample type is reported along with %CV and percent recovery range.

$$\%\,recovery = \frac{measured\ concentration}{expected\ concentration} \times 100$$

Table 5. *Spike recovery measurements of sample matrices evaluated in the Aβ Peptide Panel 1 Kits.*

Analyte	Human CSF (N=10)			Mouse Plasma (N=10)		
	Av.% Rec.	%CV	%Rec. Range	Av.% Rec.	%CV	%Rec. Range
Aβ38 (4G8)	96	5.2	90-112	90	22.0	90-112
Aβ40 (4G8)	88	11.6	70-114	100	7.2	70-114
Aβ42 (4G8)	92	11.0	79-125	100	16.5	79-125
Aβ38 (6E10)	96	6.1	85-113	NT	NT	NT
Aβ40 (6E10)	95	10.9	73-131	NT	NT	NT
Aβ42 (6E10)	95	10.7	80-132	NT	NT	NT

Av.% Rec. = Average % Recovery, %Rec. Range = % Recovery Range, NT = Not Tested

Specificity

To assess specificity, each assay in the panel was tested individually. Nonspecific binding was less than 1% for all assays in the kit. Cross-reactivity was also evaluated with a panel of related Aβ peptides and other proteins of interest, including Aβ1-16, Aβ17-24, Aβ1-37, Aβ1-39, Aβ1-41, Aβ1-43, sAPPα, sAPPβ, and Tau (Figure 3). The Aβ42 assay exhibits minor cross-reactivity with some related Aβ peptides: Aβ1-41 (2.6%); Aβ1-43 and Aβ1-37 (<0.75%). Given the low endogenous levels of these peptides in CSF, this is expected to have a minimal effect on Aβ42 quantitation.

$$\%\,nonspecificity = \frac{nonspecific\ signal}{specific\ signal} \times 100$$

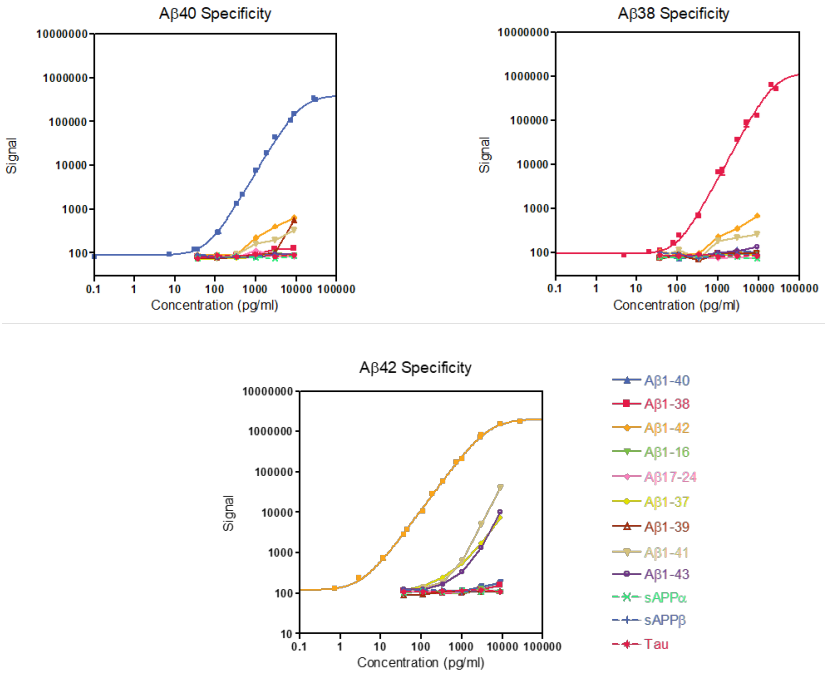


Figure 3. *Analyte specificity in the V-PLEX Aβ Peptide Panel 1 Kit.*

Interference

Aβ40 (top), Aβ38 (middle), or Aβ42 (bottom) peptide calibrators were co-spiked into assay diluents with various Aβ peptides and proteins of interest at or above expected endogenous levels for these analytes (Aβ40, sAPPα, and sAPPβ spiked at 5 ng/mL; all other interferents spiked at 1 ng/mL). Measured Aβ levels were largely within 25% of the sample with no interferent, regardless of the spiked analyte or concentration (Figure 4).

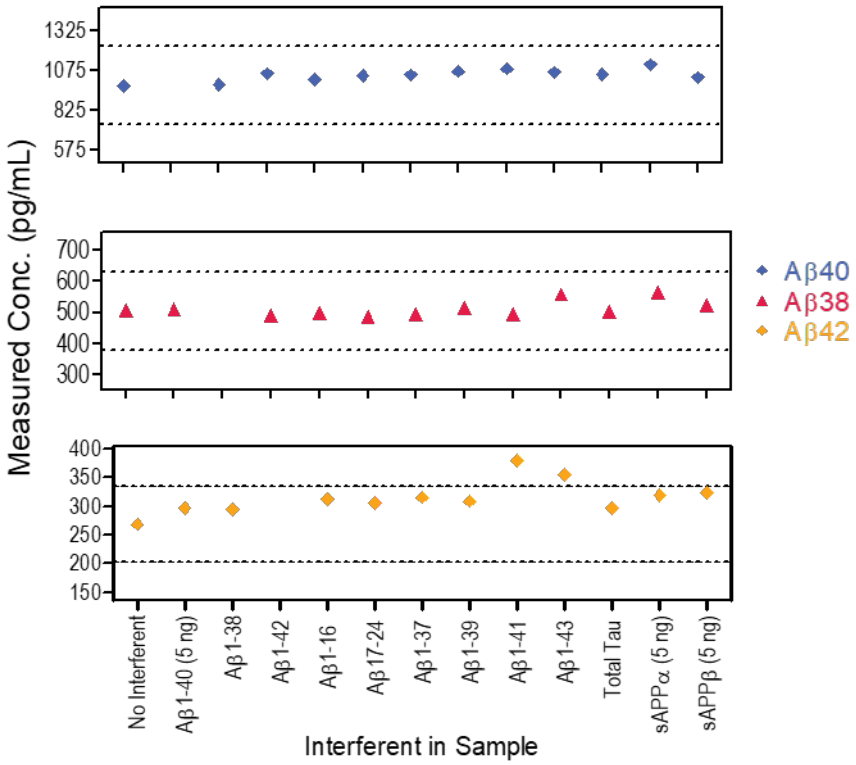
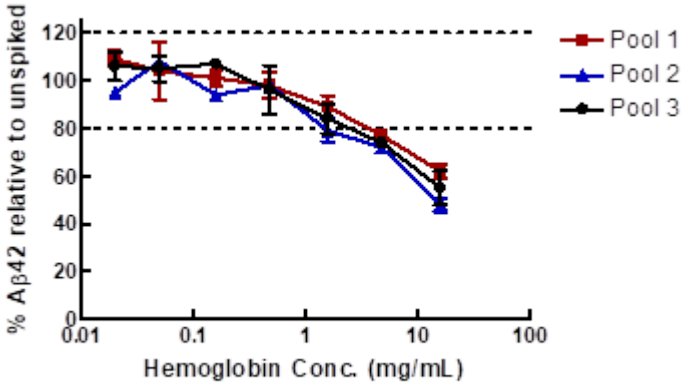


Figure 4. Measured analyte concentrations with interferents in the V-PLEX Aβ Peptide Panel 1 Kit.

Effect of Hemolysis

The Human Aβ42 Kit is tolerant of up to 1.6 mg/mL hemoglobin in CSF, which is equivalent to 1% blood contamination in the sample. Assay tolerance to blood contamination was assessed by measuring Aβ42 levels in CSF spiked with hemolyzed clarified blood. The hemoglobin concentration in the hemolyzed blood sample was estimated through absorbance measurement at 414 nm (extinction coefficient 524,280 cm^{-1}/M). The measured concentration was 160 g/L hemoglobin, consistent with expected hemoglobin levels in normal whole blood.

Hemolyzed blood was titrated into three human CSF pools. The resulting contaminated samples contained 0.02-16 mg/mL hemoglobin, which is equivalent to 0.01-10% blood in the sample. Spike samples were diluted 8-fold and tested with the Human Aβ42 Kit. The measured Aβ42 concentration relative to the unspiked sample is plotted below. Samples with 0.1% contamination are tinged slightly pink; samples with 1% contamination are dark pink and easily identified as contaminated (Figure 5).



		% Measured Concentration Relative to Unspiked Sample		
% Blood Spike Level	Hemoglobin Conc.(mg/mL)	Pool 1	Pool 2	Pool 3
10.0	16	62	48	55
3.00	4.8	77	72	74
1.00	1.6	89	79	84
0.300	0.48	98	98	96
0.100	0.16	101	94	107
0.030	0.048	104	108	105
0.010	0.016	109	95	106
0	0	100	100	100

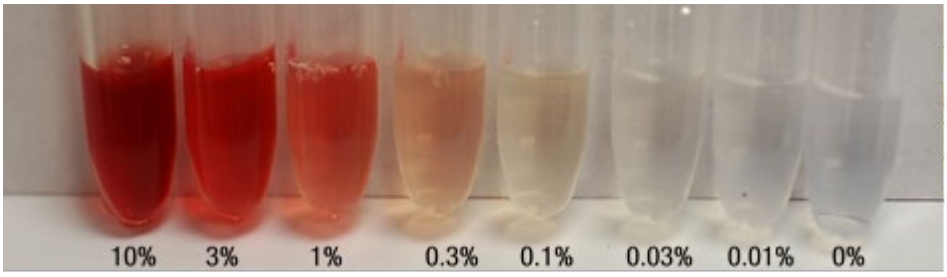


Figure 5. Effect of hemolysis on the Human Aβ42 assay in the V-PLEX Aβ Peptide Panel 1 Kit.

Assay Components

Calibrators

Table 6. Expression systems used for the calibrators in the Aβ Peptide Panel 2 Kits.

Calibrator	Source
Aβ1-38 4G8	*Synthetic Peptide*
Aβ1-40 4G8	*Synthetic Peptide*
Aβ1-42 4G8	*Synthetic Peptide*
Aβ1-38 6E10	*Synthetic Peptide*
Aβ1-40 6E10	*Synthetic Peptide*
Aβ1-42 6E10	*Synthetic Peptide*

Antibodies

Table 7. Source of the capture and detection antibodies in the Aβ Peptide Panel 2 Kits.

Antigen	Capture	Detection	Assay Generation
Aβ38 (4G8)	Recombinant Monoclonal	Recombinant Monoclonal	A
Aβ40 (4G8)	Recombinant Monoclonal	Recombinant Monoclonal	A
Aβ42 (4G8)	Recombinant Monoclonal	Recombinant Monoclonal	B
Aβ38 (6E10)	Recombinant Monoclonal	Recombinant Monoclonal	A
Aβ40 (6E10)	Recombinant Monoclonal	Recombinant Monoclonal	A
Aβ42 (6E10)	Recombinant Monoclonal	Recombinant Monoclonal	B

The Aβ 4G8 and Human Aβ 6E10 antibodies used in MSD Aβ assays are supplied by BioLegend (formerly Covance Research Products).

For Research Use Only. Not for use in diagnostic procedures.

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