Supplementary Data

Validation of Assays for Measurement of Amyloid-β Peptides in Cerebrospinal Fluid and Plasma Specimens from Patients with Alzheimer's Disease Treated with Solanezumab

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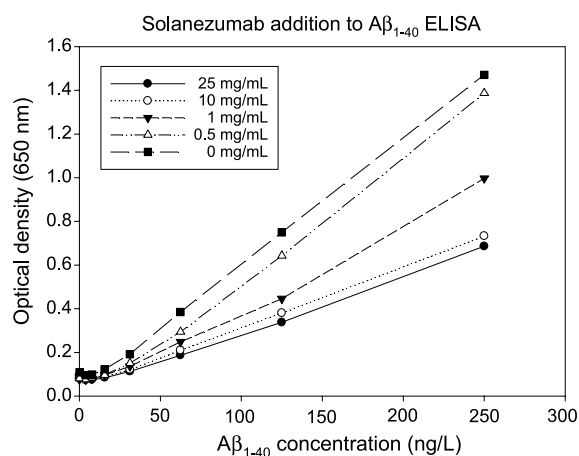[a]*Eli Lilly and Company, Windlesham, UK*
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Supplementary Figure 1. Analysis of A$\beta_{1\text{-}40}$ in rat plasma using a proprietary ELISA with different amounts of added solanezumab (see legend).
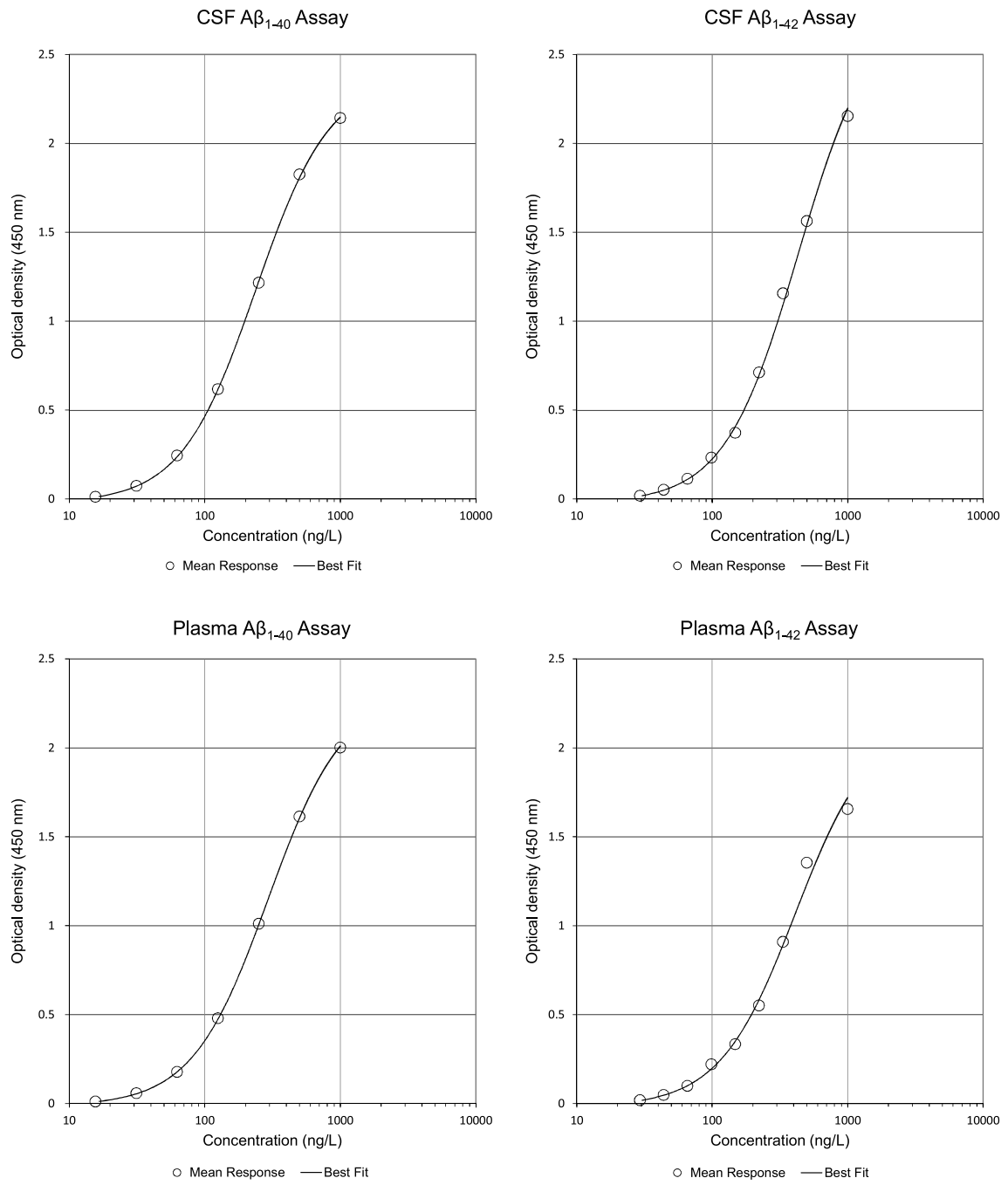
Supplementary Figure 2. Representative calibration plots for solanezumab-tolerant INNOTEST assays for quantification of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ in human cerebrospinal fluid (CSF) and plasma.
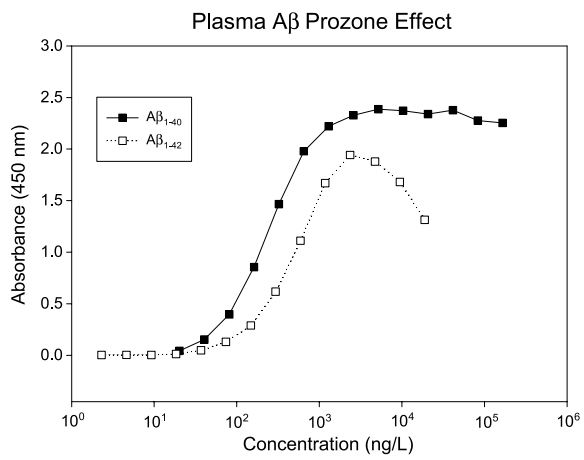
Supplementary Figure 3. Aβ plasma prozone effect. Plots of response versus Aβ concentration after 2x step-wise serial dilutions, of a plasma pool containing solanezumab with 166,000 ng/L Aβ_{1-40} and 18,900 ng/L Aβ_{1-42}, using kit diluent down to 8,192-fold. The response for Aβ_{1-42} shows a distinctive hook effect as concentration rises to a peak at 1.94 absorbance units (equivalent to 2,363 ng/L at 8x dilution).

a) CSF
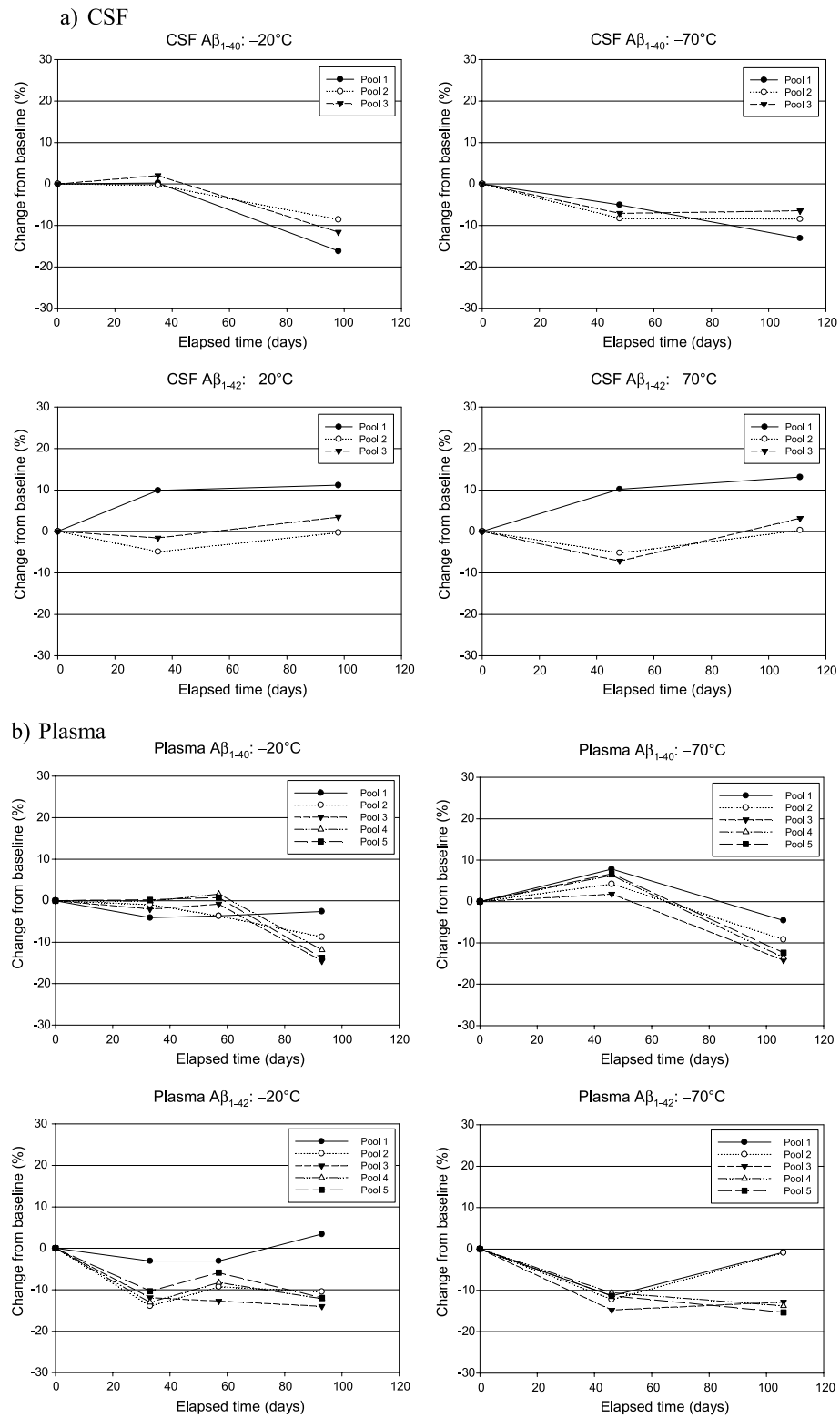
b) Plasma

Supplementary Figure 4.   Stability of Aβ peptides in stored CSF (a) and plasma (b) pools. Frozen matrix aliquots of 0.5 mL were stored at ≤–20°C or ≤–70°C. Initial pool concentration ranges (ng/L): CSF Aβ$_{1-40}$, 8,650–11,600; CSF Aβ$_{1-42}$, 547–1,190; plasma Aβ$_{1-40}$, 277–166,000; plasma Aβ$_{1-42}$, 6118,900. CSF Pool 1 and plasma Pool 1 were prepared from predose and placebo specimens.

Supplementary Table 1
Calibration performance summary for validation test batches

Calibrator level	Concentration (ng/L)								
	1	2	3	4	5	6	7	8	9
CSF Aβ_{1-40} ($n =$)	15	16	17	16	17	17	15	–	–
Theoretical concentration	15.6	31.3	62.5	125	250	500	1000	–	–
Back-calculated mean	15.6	31.2	62.8	125	248	497	1050	–	–
CV %	0.0	0.5	1.1	1.1	1.2	1.9	6.2	–	–
RE %	0.0	–0.3	0.5	0.1	–0.7	–0.6	5.0	–	–
Plasma Aβ_{1-40} ($n=$)	10	12	13	13	13	13	13	–	–
Theoretical concentration	15.6	31.3	62.5	125	250	500	1000	–	–
Back-calculated mean	15.6	31.3	62.6	126	249	493	1060	–	–
CV %	0.1	0.8	1.4	1.7	2.6	1.8	7.0	–	–
RE %	0.0	–0.1	0.1	0.5	–0.4	–1.5	5.6	–	–
CSF Aβ_{1-42} ($n =$)	15	16	17	17	17	17	17	17	17
Theoretical concentration	29.3	43.9	65.8	98.8	148	222	333	500	1000
Back-calculated mean	29.3	44.1	65.8	99.6	146	218	348	505	956
CV %	0.3	1.6	1.7	2.2	2.5	1.5	2.9	4.5	5.2
RE %	–0.1	0.6	0.0	0.8	–1.1	–1.8	4.6	1.0	–4.4
Plasma Aβ_{1-42} ($n =$)	13	13	13	13	13	13	13	13	13
Theoretical concentration	29.3	43.9	65.8	98.8	148	222	333	500	1000
Back-calculated mean	29.3	43.8	66.3	101	146	218	340	523	952
CV %	0.3	2.3	2.5	4.9	2.4	2.1	3.6	6.1	8.6
RE %	0.1	–0.3	0.7	2.0	–1.1	–1.8	2.2	4.7	–4.8

CSF, cerebrospinal fluid; CV, coefficient of variation; RE, relative error.

Supplementary Table 2
Intra-assay and inter-assay precision (as CV) and relative accuracy (as RE) summaries for repeated tests of diluent buffer validation pools

Validation pool	'CSF' assay (ng/L)							'Plasma' assay (ng/L)						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Aβ_{1-40}														
Theoretical concentration	15.6	31.3	46.8	150	500	750	1000	15.6	31.3	46.8	150	500	750	1000
Intra-assay Mean ($n=6$)	16	32	48	146	452	610	765	15[a]	30	44	141	465	672	777
CV (%)	3	3	2	1	3	4	7	2	7	3	1	4	7	7
RE (%)	2	4	1	–3	–10	–19	–24	–5	–3	–6	–6	–7	–10	–22
Inter-assay ($n =$)	11	16	16	16	16	14	14	9	10	10	10	10	10	10
Mean	16	32	48	149	484	678	889	15	29	44	147	501	700	967
CV (%)	7	5	6	4	6	7	10	9	8	3	4	6	10	25
RE (%)	4	1	2	0	–3	–10	–11	–2	–6	–5	–2	0	–7	–3
Aβ_{1-42}														
Theoretical concentration	29.3	43.9	80	150	500	750	1000	29.3	43.9	80	150	500	750	1000
Intra-assay Means ($n=6$)	31	47	87	158	522	835	1330	–	43[a]	75	146	500	807	1240
CV (%)	8	5	4	2	8	4	7		6	6	4	6	9	25
RE (%)	5	7	9	5	4	11	34	–	–3	–6	–2	0	8	24
Inter-assay ($n =$)	16	16	15	16	16	16	15	11	12	11	12	12	12	12
Mean	31	45	82	154	508	783	1100	29	43	79	150	526	780	1280
CV (%)	5	7	5	4	7	18	31	10	4	4	5	7	10	30
RE (%)	4	3	3	3	2	4	10	–2	–3	–1	0	5	4	28

CSF, cerebrospinal fluid; CV, coefficient of variation; RE, relative error. [a] Versus Inter–assay mean pool concentration; [a]$n = 5$.

Supplementary Table 3

Summary results for automation of plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ assays. Four plasma test batches were prepared using a Tecan Evo pipetting robot

	$A\beta_{1-40}$ (ng/L)				$A\beta_{1-42}$ (ng/L)			
	QC Low	QC Mid	QC High	Plasma[a]	QC Low	QC Mid	QC High	Plasma[b]
Assigned concentration	46.8	150	500	534000	80	150	500	141000
Intra-assay Means ($n = 6$)				521000[c]				140000
				469000				142000
				541000				138000
				486000[d]				141000[d]
CV (%) range				3 to 10				7 to 14
RE (%) range				−12 to 1				−2 to 1
Inter-assay Mean ($n = 6$)	48	151	458	504000[e]	72	139	503	140000[f]
CV (%)	8	4	6	8	8	7	5	10
RE (%)	3	1	−8	−6	−10	8	1	−1

QC, quality control sample; CV, coefficient of variation; RE, relative error. [a]Diluted x5000 with kit diluent; [b]Diluted x1000 with kit diluent; [c]$n = 5$; [d]Plasma centrifuged before dilution; [e]$n = 23$; [f]$n = 24$.