PRODUCT INFORMATION & MANUAL

Human Amyloid β (aa1-40) Valukine™ ELISA

Catalog Number: VAL201

For the quantitative determination of natural and recombinant human Amyloid β (aa1-40) concentrations

For research use only.
Not for diagnostic or therapeutic procedures.

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Please refer to the kit label for expiry date.
Novus kits are guaranteed for 3 months from date of receipt

Version 202410.1

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I. BACKGROUND

Amyloid beta-peptides (Aβ) are proteolytic fragments of the Amyloid Precursor Protein (APP), and their formation into fibrils plays an integral role in Alzheimer's disease pathology. Aβ fibrils constitute the major protein component of senile plaques that accumulate in the brain of Alzheimer's patients. Amyloid plaque accumulation is increased in the presence of hypoxia, traumatic brain injury, and chronic inflammation (1, 2). Aβ also directly exert multiple effects on neurons (1, 3). APP is a multifunctional transmembrane protein that consists of a 682 amino acid (aa) extracellular domain (ECD), a 24 aa transmembrane segment, and a 47 aa cytoplasmic domain. Its ECD contains two heparin binding domains, one copper binding domain, a BPTI/Kunitz inhibitor domain, and a collagen binding domain. Alternative splicing generates multiple isoforms including the most prevalent APP695, APP751, and APP770 which contain various deletions or substitutions in the ECD. The Aβ peptide sequences extend from the extracellular juxtamembrane region into the transmembrane segment.

APP is subject to proteolytic cleavage at multiple sites that may or may not result in the generation of Aβ. In the non-amyloidogenic pathway, the APP ECD is shed by β-secretase cleavage (ADAM10) within the Aβ sequence, thereby preventing the formation of Aβ peptides (4). In the amyloidogenic pathway, APP is cleaved by BACE (β-secretase activity) just N-terminal to the Aβ sequence (5, 6). This releases a slightly shorter ECD fragment known as APPbeta. Following amyloidogenic ECD shedding, intramembrane cleavage of the remaining portion by γ-secretase/Presenilin protein complexes generates the Aβ peptides 1-40 or 1-42 (7-9). These peptides are 40 and 42 aa in length, respectively, and share Asp672 as their N-terminal residue. Aβ 1-40 is relatively abundant in the CSF, while Aβ 1-42 is more hydrophobic, more prone to aggregation, and more closely associated with Alzheimer's pathology (1, 10). Human Aβ 1-40 shares 93% amino acid identity with mouse and rat Aβ 1-40. γ-secretase cleavage also releases the intracellular domain (AICD) which translocates to the nucleus and regulates the transcription of a wide variety of genes (11). Soluble fragments of the APP ECD are released into the cerebrospinal fluid (CSF), interstitial fluid in brain, plasma, and urine (12-15). They can be further cleaved to generate a 35 kDa fragment which binds the receptor DR6 and triggers axonal pruning and neurodegeneration (16).

Aβ peptides can associate intracellularly into dimers, higher order oligomers, and insoluble fibrils (17-19). They enhance the hyperphosphorylation of Tau and the formation of neurofibrillary tangles, another hallmark of Alzheimer's disease (20). Aβ also form fibrils with ApoE and bind to the prion protein PrP(C) (21, 22). Of the various forms of Aβ, oligomers are the most potent at impairing cognitive function, inhibiting long term potentiation (LTP) in hippocampal neurons, and inducing neuronal oxidative

stress (17, 23, 24). In contrast, monomeric Aβ can be protective against excitotoxic neuronal cell death (25). Aβ can directly disrupt the integrity of neuronal membranes (26). The neurotoxicity of Aβ is increased by its in vivo glycation or pyroglutamylation (27, 28). Aβ interaction with RAGE induces neuronal mitochondrial dysfunction, oxidative stress, inflammatory activation of astrocytes and microglia, and transport of circulating Aβ across the blood brain barrier into the brain (29-31). Aβ also induces neuroinflammation through interactions with TLR2 (32). Extracellular Aβ can be degraded by Neprilysin or Insulysin/IDE, and Aβ clearance is impaired in Alzheimer's (33-36).

Aβ peptides exert direct actions on several neurotransmitter receptors, and APP metabolism is regulated by the activity of these receptors. Aβ bind and activate the nicotinic acetylcholine alpha 7 receptor, leading to neuroprotection, enhanced synaptic plasticity and memory formation, and the upregulation of acetylcholinesterase (37-39). Aβ activation of NMDA and AMPA receptors, however, leads to mitochondrial dysfunction and neuronal apoptosis (40). Aβ inhibit the release of dopamine and GABA in response to muscarinic receptor activation (41). The non-amyloidogenic processing of APP is induced by multiple triggers including Substance P or the activation of particular subtypes of muscarinic, nicotinic, purinergic, serotonin, NMDA, or metabotropic glutamate receptors (42-48).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human Amyloid β (aa1-40) has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human Amyloid β (aa1-40) present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for human Amyloid β (aa1-40) is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, TMB substrate (Chromogenic agent) is added to the wells and color develops in proportion to the amount of human Amyloid β (aa1-40) bound in the initial step. The color development is stopped, and the intensity of the color is measured.

B. LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- This kit is suitable for cell culture supernate, tissue lysates and cerebrospinal fluid.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with Diluent RD2-7 and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

III. ADVANTAGES

A. PRECISION

Intra-assay Precision (Precision within an assay)

Three samples were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples were tested in twenty separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
Mean (pg/mL)	58.9	264	556	56.3	247	518
Standard Deviation	4.23	5.76	11.3	4.31	22.4	30.5
CV%	7.2	2.2	2.0	7.7	9.1	5.9

B. RECOVERY

The recovery of human Amyloid β (aa1-40) spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range (%)
Cell culture media (n=4)	99	95-105
Cell Lysis Buffer (n=2)	99	91-110
Cerebrospinal fluid* (n=4)	104	96-113

*Samples were diluted prior to assay as directed in the Sample Preparation section.

C. SENSITIVITY

Twenty-six assays were evaluated and the minimum detectable dose (MDD) of human Amyloid β (aa1-40) ranged from 1.31-8.17 pg/mL. The mean MDD was 3.97 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

D. CALIBRATION

This immunoassay is calibrated against synthetic human Amyloid β (aa1-40).

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of human Amyloid β (aa1-40) and diluted with Diluent RD2-7 to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture media (n=4)	Cell lysis buffer (n=2)	Cerebrospinal fluid (n=4)
1:2	Average % of Expected	98	99	102
	Range (%)	95-100	98-101	97-110
1:4	Average % of Expected	95	98	104
	Range (%)	93-98	93-104	99-114
1:8	Average % of Expected	96	101	105
	Range (%)	92-100	94-108	97-118
1:16	Average % of Expected	95	97	100
	Range (%)	87-101	88-106	85-110

F. SAMPLE VALUES

Note: *All samples were handled on ice.*

Cerebrospinal Fluid - Purchased samples were evaluated for the presence of human Amyloid β (aa1-40) in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Cerebrospinal fluid (n=60)	5875	95	ND-15,708

ND=Non-detectable

Cell culture supernates:

Peripheral blood leukocytes (1 × 10^6 cells) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum. Cells were cultured unstimulated or stimulated with 10

µg/mL PHA for 1 and 5 days. Aliquots of the cell culture supernates were removed and assayed for human Amyloid β (aa1-40).

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	42.2	ND
Stimulated	39.6	31.3

ND=Non-detectable

IMR-32 human neuroblastoma cells were cultured in MEM supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate until confluent. An aliquot of the cell culture supernate was removed, assayed for human Amyloid β (aa1-40), and measured 102 pg/mL.

Tissue Lysates - Tissues were rinsed with PBS, cut into 1-2 mm pieces, and homogenized with a tissue homogenizer in PBS. An equal volume of RIPA buffer containing protease inhibitors was added and tissues were lysed on ice for 30 minutes with gentle agitation. Debris was then removed by centrifugation. Aliquots of the lysates were removed and assayed for human Amyloid β (aa1-40).

Tissue	pg/mL
Human hippocampus	363
Human inner cortex	513
Human motor cortex	49.9
Human outer cortex	3980

G. SPECIFICITY

This assay recognizes natural and recombinant human Amyloid β (aa1-40).

The factors listed below were prepared in Diluent RD2-7 and assayed for cross-reactivity. Preparations of the following factors in a mid-range recombinant human Amyloid β controlwere assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human：	**Synthetic proteins:**
Amyloid Precursor Protein	human Amyloid β (aa4-40)
Apolipoprotein E2	human Amyloid β (aa17-40)
Apolipoprotein E3	human Amyloid β (aa22-40)
Apolipoprotein E4	mouse/rat Amyloid β (aa1-40)
	mouse/rat Amyloid β (aa1-42)

Synthetic human Amyloid β (aa2-40) cross-reacts approximately 11%.

Synthetic human Amyloid β (aa3-40) cross-reacts approximately 1.5%.

Synthetic human Amyloid β (aa1-42), (aa1-43), (aa1-44), and Amyloid β [Pyr3] (aa3-40) cross-react ≤ 0.2%.

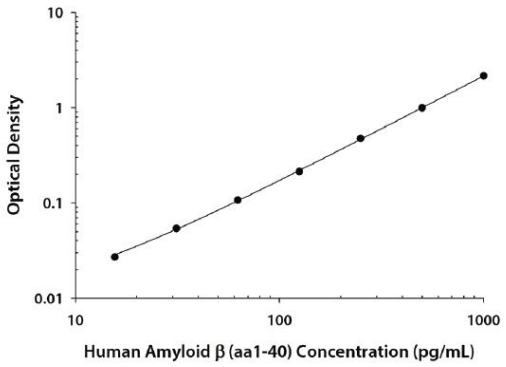
Synthetic human Amyloid β [Pyr11] (aa11-40) does not cross-react in this assay but does interfere at concentrations > 20 ng/mL.

Synthetic human Amyloid β (aa11-40) does not cross-react but does interfere at concentrations > 12.5 ng/mL.

IV. EXPERIMENT

EXAMPLE STANDARD

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.027 0.031	0.029	—
15.6	0.052 0.059	0.056	0.027
31.3	0.082 0.084	0.083	0.054
62.5	0.131 0.140	0.136	0.107
125	0.236 0.247	0.242	0.213
250	0.493 0.511	0.502	0.473
500	1.016 1.025	1.021	0.992
1000	2.166 2.194	2.180	2.151

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human Amyloid β (aa1-40) Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human Amyloid β (aa1-40)	1 plate
Human Amyloid β (aa1-40) Conjugate	Solution of antibody against human Amyloid β (aa1-40) conjugated to horseradish peroxidase	1 vial
Human Amyloid β (aa1-40) Standard	Synthetic human Amyloid β (aa1-40) peptide in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume	2 vials
Diluent RD2-7	A buffered protein base used to dilute standard and samples	2 vials
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant	1 vial
TMB Substrate	TMB ELISA Substrate Solution/TMB Substrate Solution	2 vials
Stop Solution	2 N sulfuric acid	1 vial
Plate Sealers	Adhesive strip	3 strips

B. STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2-8°C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="6">Opened/ Reconstituted Reagents</td><td>Wash Buffer (1×)</td><td rowspan="4">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Standard</td><td>Use a new standard for each assay.
Discard after use.</td></tr>
<tr><td>Diluent RD2-7</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8°C.*</td></tr>
</table>

* Provided this is within the expiration date of the kit.

C. OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinder.

D. PRECAUTION

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Note: *Thaw samples on ice to prevent aggregation. Avoid repeated freeze-thaw cycles.*

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Samples may require dilution with Diluent RD2-7.

Tissue Lysates - Lysates were prepared as described in the Sample Value section. Samples may require dilution with Diluent RD2-7.

Cerebrospinal Fluid - Freeze sample within one hour of collection to avoid aggregation. Store samples at ≤ -70 °C. Samples may require dilution with Diluent RD2-7.

Note: *Amyloid β is labile in cerebrospinal fluid and can become undetectable over time.*

B. SAMPLE PREPARATION

Use polypropylene tubes.

Tissue lysate samples recommend a 40-fold dilution. A suggested 40-fold dilution is 20 µL of sample + 780 µL of Diluent RD2-7. Optimal dilutions should be determined by the end user.

Cerebrospinal fluid samples recommend a minimum 20-fold dilution. A suggested 20-fold dilution is 30 µL of sample + 570 µL of Diluent RD2-7. Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Note: Bring all reagents to room temperature before use.

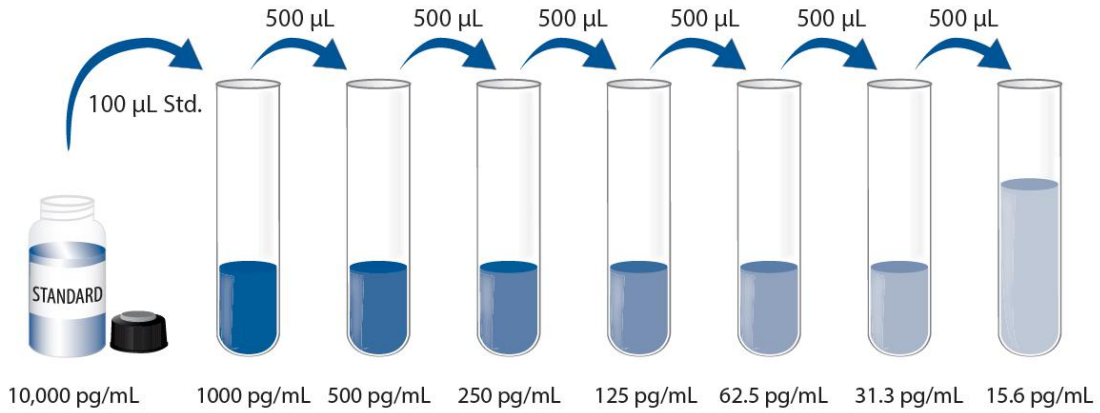
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

Human Amyloid β (aa1-40) Standard - Refer to the vial label for the reconstitution volume* Reconstitute the Human Amyloid β (aa1-40) Standard with deionized or distilled water. This reconstitution produces a stock solution of 10000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation

prior to making dilutions.

*If you have any question, please seek help from our Technical Support.

Use polypropylene tubes. Pipette 900 µL of Diluent RD2-7 into the 1000 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1000 pg/mL standard serves as the high standard. Diluent RD2-7 serves as the zero standard (0 pg/mL).



D. TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- TMB Substrate should remain colorless until added to the plate. Keep TMB Substrate protected from light. TMB Substrate should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the TMB Substrate. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB Substrate.

VII.ASSAY PROCEDURE

Note: **The Human Amyloid β (aa1-40) Conjugate must remain at 2-8 °C during use. Bring all other reagents and samples to room temperature before use. It is recommended that all standards and samples be assayed in duplicate.**

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 200 µL of standard and prepared sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at 2-8 °C.** A plate layout is provided for a record of standards and samples assayed.
4. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 200 µL of **cold** human Amyloid β (aa1-40) Conjugate to each well. Cover with a new adhesive strip. **Incubate for 2 hours at 2-8 °C.**
6. Repeat the aspiration/wash as in step 4.
7. Add 200 µL of TMB Substrate to each well. **Incubate for 30 minutes at room temperature. Protect from light**.
8. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.
10. **CALCULATION OF RESULTS**

    Average the duplicate readings for each standard and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human Amyloid β

(aa1-40) concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

VIII. REFERENCES

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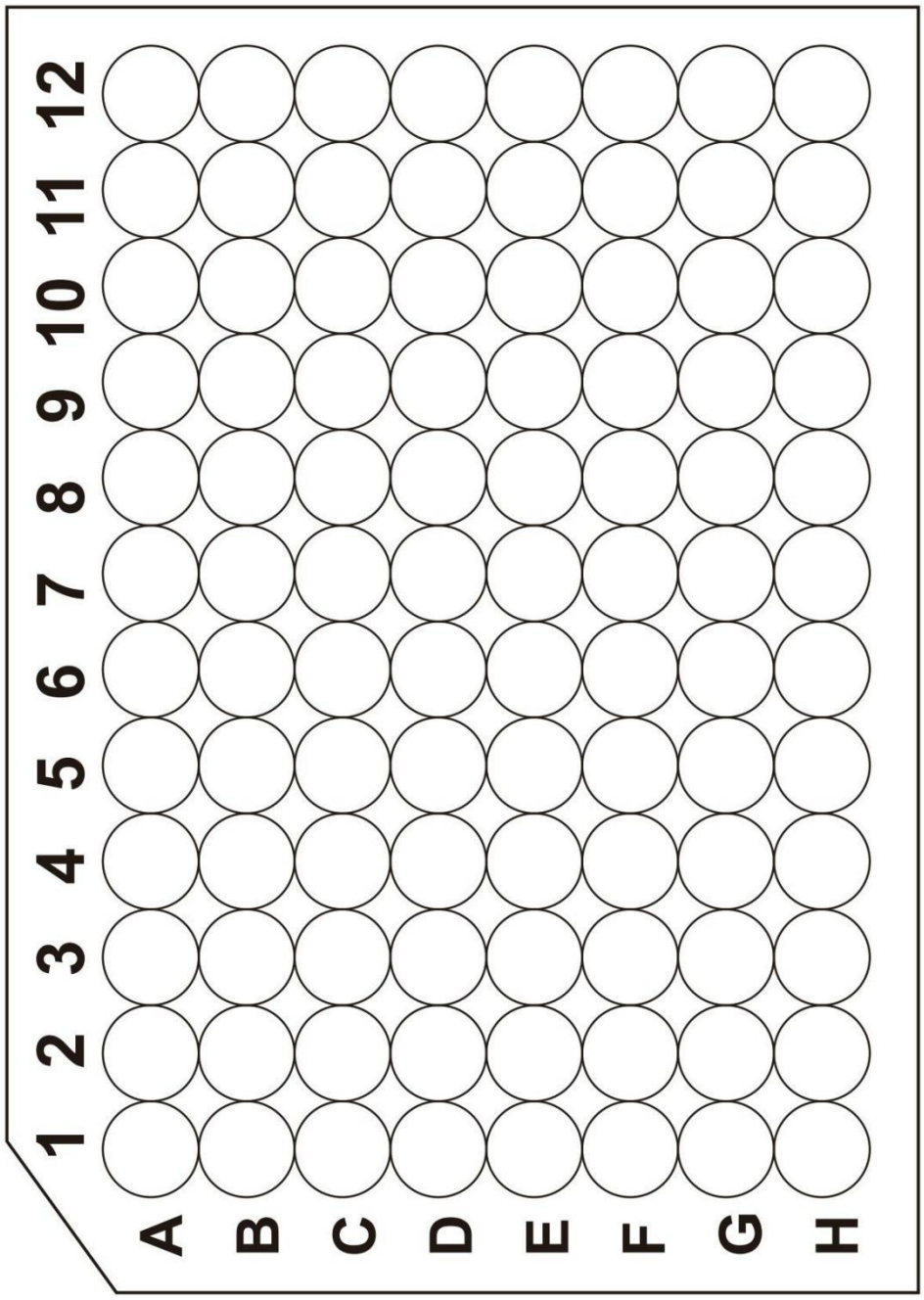
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人淀粉样蛋白β (aa1-40) Valukine™ ELISA 试剂盒

目录号：VAL201

适用于定量检测天然和重组人淀粉样蛋白β (aa1-40)的浓度

科研专用，不可用于临床诊断

Bio-Techne China Co. Ltd

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版本号 202410.1

目录

I. 背景

淀粉样β肽（Amyloid beta-peptides , Aβ）是淀粉样蛋白前体蛋白（Amyloid Precursor Protein,APP）的蛋白水解片段，它们成纤维的形成在阿尔茨海默氏病病理学中起着不可或缺的作用。Aβ纤维是老年痴呆症患者脑内老年斑的主要蛋白质成分。缺氧、脑外伤和慢性炎症会增加淀粉样蛋白斑块的积累（1, 2）。Aβ还直接对神经元发挥多种影响（1，3）。APP 是一种多功能跨膜蛋白，由一个 682 氨基酸(amino acid ,aa)的胞外结构域(extracellular domain,ECD)、一个 24 aa的跨膜区段和一个 47 aa的胞浆结构域组成。其 ECD 包含两个肝素结合结构域、一个铜结合结构域、一个 BPTI/Kunitz 抑制剂结构域和一个胶原蛋白结合结构域。替代剪接产生多种异构体，包括最常见的 APP695、APP751 和 APP770，这些异构体在 ECD 中含有各种缺失或替换。Aβ肽序列从细胞外并膜区延伸到跨膜区。APP 可在多个位点发生蛋白水解，从而产生或不产生Aβ。在非淀粉样蛋白生成途径中，APP的ECD被Aβ序列内的β-分泌酶（ADAM10）裂解脱落，从而阻止了Aβ肽的形成（4）。在淀粉样蛋白生成途径中，APP 在Aβ序列的 N 端被β-分泌酶活性（BACE）裂解（5，6）这会释放出一个稍短的 ECD 片段，即 APPbeta。淀粉样变性 ECD 脱落后，γ-分泌酶/早老素 蛋白复合物对剩余部分进行膜内裂解，生成Aβ肽 1-40 或 1-42（7-9）。这些肽的长度分别为 40 和 42 aa，其 N 端残基均为Asp672。在CSF中，Aβ1-40相对丰富，而Aβ1-42更疏水，更容易聚集，并且与阿尔茨海默氏病的病理密切相关（1，10）。Aβ1-40 在脑脊液（cerebrospinal fluid ，CSF）中含量相对较高，而Aβ1-42 疏水性更强，更容易聚集，与阿尔茨海默病的病理变化关系更为密切（1, 10）。人类Aβ1-40 与小鼠和大鼠Aβ1-40 有 93% 的氨基酸相同性。γ-分泌酶裂解还释放出细胞内结构域（AICD），该结构域可转位至细胞核并调节多种基因的转录 (11)。APP ECD 的可溶性片段会释放到CSF、脑间质、血浆和尿液中（12-15）。它们可被进一步裂解，生成 35 kDa 的片段，与受体 DR6 结合，引发轴突修剪和神经变性（16）。

Aβ肽可在细胞内结合成二聚体、高阶寡聚体和不溶性纤维（17-19）。它们会增强 Tau 的过度磷酸化和神经纤维缠结的形成，这是阿尔茨海默病的另一个特征（20）。Aβ还会与载脂蛋白形成纤维，并与朊病毒蛋白 PrP(C) 结合（21，22）。在各种形式的 Aβ中，低聚物在损害认知功能、抑制海马神经元的长期增强作用（long term potentiation ，LTP）和诱导神经元氧化应激方面的作用最强（17、23、24）。相反，单体Aβ对兴奋性毒性神经元细胞死亡具有保护作用（25）。Aβ可直接破坏神经元膜的完整性（26）。体内糖化或焦谷氨酰化会增加Aβ的神经毒性（27，28）。Aβ与 RAGE 的相互作用诱导神经元线粒体功能障碍、氧化应激、星形胶质细胞和小胶质细胞的炎症激活，以及循环Aβ穿过血脑屏障进入大脑（29-31）。Ab 还会通过与 TLR2 的相互作用诱发神经炎症（32）。细胞外Aβ可被脑啡肽酶或 胰岛素溶酶/胰岛素降解酶降解，阿尔茨海默氏症患者的Aβ清除能力受损（33-36）。

Aβ肽直接作用于几种神经递质受体，APP 的代谢受这些受体活性的调节。Aβ可结合并激活烟碱乙酰胆碱α7受体，从而产生神经保护作用，增强突触可塑性和记忆形成，并上调乙酰胆碱酯酶（37-39）。然而Aβ激活NMDA和AMPA受体会导致线粒体功能障碍和神经元凋亡（40）。Aβ可抑制毒蕈碱受体激活时多巴胺和GABA的释放（41）。APP 的非淀粉样蛋白生成处理由多种触发因素诱导，包括神经肽P物质或毒蕈碱、烟碱、噻吟能、血清素、NMDA 或代谢谷氨酸受体特定亚型的激活（42 -48）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人淀粉样蛋白β (aa1-40) 抗体包被于微孔板上，样品和标准品中的人淀粉样蛋白β (aa1-40)会与固定在板上的抗体结合，游离的成分被洗去；加入辣根过氧化酶标记的抗人淀粉样蛋白β (aa1-40)检测抗体进行孵育。洗涤去除未结合的试剂后，加入TMB底物溶液（显色剂）。溶液颜色与结合目标蛋白成正比；加入终止液；用酶标仪测定吸光度。

B. 检测局限

- **仅供科研使用，不可用于体外诊断；**
- 该试剂盒适用于细胞培养上清样本，组织裂解物和脑脊液；
- 请在试剂盒有效期内使用；
- 不同试剂盒及不同批号试剂盒的组分不能混用；
- 样本值若大于标准曲线的最高值，应将样本用稀释液RD2-7稀释后重新检测；
- 检测结果的不同可由多种因素引起，包括实验人员的操作、移液器的使用方式、洗板技术、反应时间或温度、试剂盒的效期等。

III. 优势

A. 精确度

板内精确度（同一板内不同孔间的精确度）

已知浓度的三个样本，在同一板内分别检测20次，以确定板内精确度。

板间精确度（不同板之间的精确度）

已知浓度的三个样本，在不同板间分别检测20次，以确定板间精确度。

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (pg/mL)	58.9	264	556	56.3	247	518
标准差	4.23	5.76	11.3	4.31	22.4	30.5
CV%	7.2	2.2	2.0	7.7	9.1	5.9

B. 回收率

不同类型样本中掺入检测范围内不同水平的人淀粉样蛋白β (aa1-40)，测定其回收率。

样本类型	平均回收率 (%)	范围 (%)
细胞培养基 (n=4)	99	95-105
细胞裂解液 (n=2)	99	91-110
脑脊液* (n=4)	104	96-113

*按照样品制备部分的指示，在测定前稀释样品。

C. 灵敏度

进行26次检测评估，人淀粉样蛋白β (aa1-40)的最低可检测剂量（MDD）范围为1.31-8.17 pg/mL。平均MDD为3.97 pg/mL。

MDD是根据20个重复的零标准品孔的吸光度值的平均值加两倍标准差计算得到的相对应浓度。

D. 校正

该免疫检测法以合成人淀粉样蛋白β (aa1-40)校正。

E. 线性

不同的样本中含有或掺入高浓度的人淀粉样蛋白β (aa1-40)，然后用稀释液RD2-7将样本稀释到检测范围内，测定其线性。

稀释倍数		细胞培养基 (n=4)	细胞裂解液 (n=2)	脑脊液 (n=4)
1:2	平均值/期待值（%）	98	99	102
	范围 (%)	95-100	98-101	97-110
1:4	平均值/期待值（%）	95	98	104
	范围 (%)	93-98	93-104	99-114
1:8	平均值/期待值（%）	96	101	105
	范围 (%)	92-100	94-108	97-118
1:16	平均值/期待值（%）	95	97	100
	范围 (%)	87-101	88-106	85-110

F. 样本预值

注意: *所有样本需要在冰上处理.*

脑脊液-在本试验中评估了购买的样本中是否存在人淀粉样β（aa1-40）。本研究中使用的供体没有病史。

样本类型	平均检测值 (pg/mL)	%可检测率	范围 (pg/mL)
脑脊液 (n=60)	5875	95	ND-15,708

ND=未检测出

细胞培养上清：

人的外周血单核细胞（1×10^6 细胞/mL）培养于含有10%胎牛血清的RPMI1640培养基中。细胞在未刺激或用10 μg/mL PHA刺激的情况下培养1天和5天。取出细胞培养上清液的等分试样，并测定人淀粉样蛋白β（aa1-40）。

条件	1天 (pg/mL)	5天 (pg/mL)
未刺激	42.2	ND
刺激	39.6	31.3

ND=未检出

IMR-32人神经母细胞瘤细胞在补充有10%胎牛血清、1 mM丙酮酸钠、2 mM L-谷氨酰胺、100 U/mL青霉素和100 µg/mL硫酸链霉素的MEM中培养，直至融合。取出一份细胞培养上清液，测定人淀粉样蛋白β（aa1-40），测量值为102 pg/mL。

组织裂解物-用PBS冲洗组织，切成1-2 mm的小块，在PBS中用组织匀浆器均质化。加入等体积的含有蛋白酶抑制剂的RIPA缓冲液，在冰上缓慢搅拌30分钟，溶解组织。然后通过离心去除碎片。取出裂解物的等分试样，并测定人淀粉样蛋白β（aa1-40）。

组织	pg/mL
人海马体	363
人体内皮层	513
人类运动皮层	49.9
人类外皮质	3980

ND=未检测出

G. 特异性

检测方法识别天然和重组的人淀粉样蛋白β（aa1-40）。

以下列出的因子是在稀释液RD2-7中制备的，并进行了交叉反应性测定。在中值浓度重组人淀粉样蛋白β对照品中制备以下因子来进行了干扰检测。未观察到明显的交叉反应或干扰。

Recombinant human：	Synthetic proteins:
Amyloid Precursor Protein	human Amyloid β (aa4-40)
Apolipoprotein E2	human Amyloid β (aa17-40)
Apolipoprotein E3	human Amyloid β (aa22-40)
Apolipoprotein E4	mouse/rat Amyloid β (aa1-40)
	mouse/rat Amyloid β (aa1-42)

合成的人淀粉样蛋白β（aa2-40）交叉反应约为11%。

合成的人淀粉样蛋白β（aa3-40）交叉反应约为1.5%。

合成的人淀粉样β（aa1-42）、（aa1-43）、（aa1-44）和淀粉样β [Pyr3]（aa3-40）的交叉反应≤0.2%。

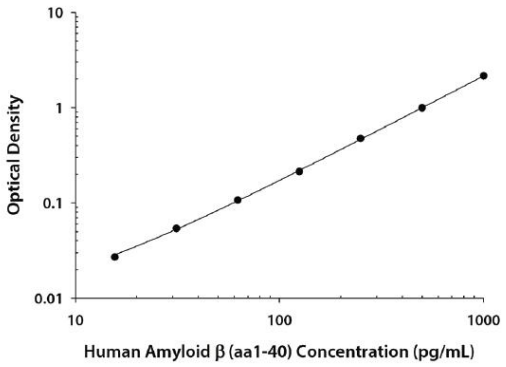
合成的人淀粉样β [Pyr11]（aa11-40）在本试验中不发生交叉反应，但在浓度>20 ng/mL时会产生干扰。

合成的人淀粉样蛋白β（aa11-40）不会发生交叉反应，但在浓度>12.5 ng/mL时会发生干扰。

IV. 实验

标准曲线实例

该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。



(pg/mL)	O.D.	Average	Corrected
0	0.027 0.031	0.029	—
15.6	0.052 0.059	0.056	0.027
31.3	0.082 0.084	0.083	0.054
62.5	0.131 0.140	0.136	0.107
125	0.236 0.247	0.242	0.213
250	0.493 0.511	0.502	0.473
500	1.016 1.025	1.021	0.992
1000	2.166 2.194	2.180	2.151

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human Amyloid β (aa1-40) Microplate	包被抗人淀粉样蛋白β（aa1-40）抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Human Amyloid β (aa1-40) Conjugate	酶标检测抗人淀粉样蛋白β（aa1-40）抗体	1瓶
Human Amyloid β (aa1-40) Standard	合成的人淀粉样蛋白β（aa1-40）肽标准品（冻干），参考瓶身标签进行重溶	2瓶
Diluent RD2-7	稀释液用于稀释标准品和样本	2瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1瓶
TMB Substrate	TMB ELISA底物溶液/TMB底物溶液	2瓶
Stop Solution	终止液	1瓶
Plate Sealers	封板膜	3张

B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8℃储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="6">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="4">2-8℃储存，最多30天*</td></tr>
<tr><td>终止液</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>TMB底物溶液</td></tr>
<tr><td>标准品</td><td>每次用新的标准品，用完丢弃</td></tr>
<tr><td>稀释液RD2-7</td><td>2-8℃储存，最多30天*</td></tr>
<tr><td>包被的微孔板条</td><td>将未用的板条放回带有干燥剂的铝箔袋内，密封；2-8 ℃储存，最多30天*</td></tr>
</table>

*必须在试剂盒有效期内

C. 实验所需自备试验器材

- 酶标仪（可测量450 nm检测波长的吸收值及540 nm或570 nm校正波长的吸收值）
- 高精度加液器及一次性吸头
- 蒸馏水或去离子水
- 洗瓶（喷瓶）、多通道洗板器或自动洗板机
- 100 mL和500 mL量筒

D. 注意事项

- 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，避免吸入。
- 试剂盒中的终止液是酸性溶液，使用时请做好眼睛、手、面部及衣服的防护。

VI. 实验前准备

A. 样品收集及储存

以下列出的样品收集和储存条件仅作为一般指南。样品稳定性尚未评估。

注意： *在冰上解冻样品以防止聚集。避免反复冻融循环。*

细胞培养上清液-通过离心去除颗粒物，立即或等分进行检测，并将样品储存在≤-20°C的温度下。样品可能需要用稀释液RD2-7稀释。

组织裂解物-按照样品值部分所述制备裂解物。样品可能需要用稀释液RD2-7稀释。

脑脊液-在采集后一小时内冷冻样本，以避免聚集。将样品储存在≤-70°C的温度下。样品可能需要用稀释液RD2-7稀释。

注： *淀粉样蛋白β在脑脊液中不稳定，随着时间的推移可能无法检测到。*

B. 样本准备工作

使用聚丙烯管。

组织裂解物样本建议稀释40倍。建议的40倍稀释度为20 µL样品+780 µL稀释液RD2-7。最佳稀释度应由最终用户确定。

脑脊液样本建议至少稀释20倍。建议的20倍稀释度为30 µL样品+570 µL稀释液RD2-7。最佳稀释度应由最终用户确定。

C. 检测前准备工作

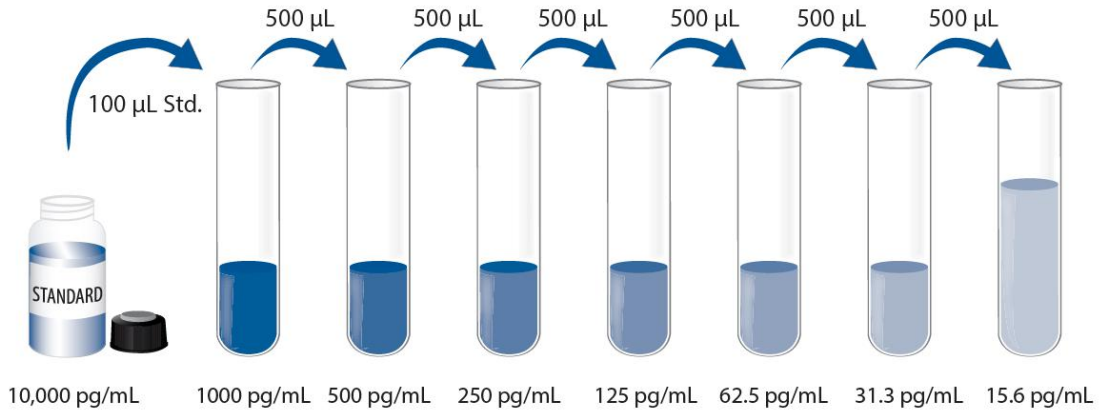
注意： ***使用前请将所有试剂放置于室温。***

洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液（25×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

人淀粉样蛋白β（aa1-40）标准品：重溶体积请参考瓶身标签*，用去离子水或蒸馏水重构人淀粉样蛋白β（aa1-40）标准品，得到浓度为10000 pg/mL标准品储备母液。轻轻震摇至少15分钟，其充分溶解。

*如有疑问，请咨询我们的技术支持。

使用聚丙烯管。用移液管将900 µL稀释液RD2-7移入1000 pg/mL管子中。用移液管吸取500 µL至其余管子中。使用储备母液溶液稀释（如下）。在下次转移之前，将每根管彻底混合。1000 pg/mL作为标准品最高点。稀释液RD2-7作为标准品零点（0 pg/mL）。



D. 技术小提示

- 当混合或重溶蛋白液时，尽量避免起沫；
- 为了避免交叉污染，配制不同浓度标准品、上样、加不同试剂都需要更换枪头。另外不同试剂请分别使用不同的移液槽；
- 建议15分钟内完成一块板的上样；
- 每次孵育时，正确使用封板膜可保证结果的准确性；
- TMB底物溶液在上板前应为无色，请避光保存；加入微孔板后，将由无色变成不同深度的蓝色；
- 终止液上板顺序应同TMB底物溶液上板顺序一致；加入终止液后，孔内颜色由蓝变黄； 若孔内有绿色，则表明孔内液体未混匀请充分混合

VII. 操作步骤

注意：人淀粉样蛋白β（aa1-40）酶标结合物在使用过程中必须保持在2-8°C。使用前，将所有其他试剂和样品带至室温。建议对所有标准品和样品进行复孔检测。

1. 按照上一节的说明，准备好所有需要的试剂和标准品；
2. 从已平衡至室温的密封袋中取出微孔板，未用的板条请放回铝箔袋内，重新封口；
3. 分别将不同浓度标准品和实验样本加入相应孔中，每孔200 µL。用封板膜封住反应孔，**2-8 °C孵育2小时**。说明书提供了一张96孔模板图，可用于记录标准品和试验样本的板内位置；
4. 将板内液体吸去，使用洗瓶、多通道洗板器或自动洗板机洗板。每孔加洗涤液400 µL，然后将板内洗涤液吸去。重复操作2次，共洗3次。每次洗板尽量吸去残留液体会有助于得到好的实验结果。最后一次洗板结束，请将板内所有液体吸干或将板倒置，在吸水纸拍干所有残留液体；
5. 在每个微孔内加入**冷的**200 µL人淀粉样蛋白β（aa1-40）酶标检测抗体。用封板膜封住反应孔，**2-8 °C孵育2小时**；
6. 重复第4步洗板操作；
7. 在每个微孔内加入200 µL TMB底物溶液，**室温孵育30分钟**。注意避光；
8. 在每个微孔内加入50 µL终止液，请轻拍微孔板，使溶液混合均匀；
9. 加入终止液后10分钟内，使用酶标仪测量450 nm的吸光度值，设定540 nm或570 nm作为校正波长。如果波长校正不可用，以450 nm的读数减去540 nm或570 nm的读数。这种减法将校正酶标板上的光学缺陷。没有校正而直接在450 nm处进行的读数可能会更高且更不准确；
10. 计算结果：

    将每个标准品和样品的复孔吸光值取平均值，然后减去零标准品平均OD值（O.D.），使用计算机软件作四参数逻辑（4-PL）曲线拟合创建标准曲线。另一替代方法是，通过绘制y轴上每个标准品的平均吸光值与x轴上的浓度来构建标准曲线，并通过图上的点绘制最佳拟合曲线。数据可以通过绘制人淀粉样蛋白β（aa1-40）浓度的对数与O.D.的对数来线性化，并且最佳拟合线可以通过回归分析来确定。该程序将产生足够但不太精确的数据拟合。

    如果样品被稀释，从标准曲线读取的浓度必须乘以稀释倍数。

VIII. 参考文献

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96 孔模板图

请使用 96 孔模板图来记录标准品及样本在板内的位置