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biosensis® Oligomeric Amyloid-β (o-Aβ) ELISA Kit

Catalog Number: BEK-2215-1P/2P

For the detection of human β-amyloid oligomer complexes in human CSF, brain tissue extracts and human transgenic mouse samples only if used as directed.

Please refer to the Sample Preparation Section for specific use instructions for each substrate application.

This ELISA kit now includes a pre-formed, oligomerized human Aβ42 peptide calibrator standard for increased reproducibility. Reagents from previous versions of this ELISA kit should not be used.

For research use only, not for use in clinical and diagnostic procedures.

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1. Intended Use

The purpose of this kit is the detection of human β-amyloid oligomer complexes in human CSF, brain tissue extracts and human transgenic mouse samples only if used as directed. This kit has not been tested for other sample applications. Non-validated samples may contain immunoglobulins and other components that potentially interfere with this ELISA assay. Biosensis does not assume responsibility if this kit is used for unintended purposes.

For research use only. Not for diagnostic and clinical purposes.

2. Introduction

The oligomeric form of Amyloid Beta peptide (Aβ, 1-42) has been closely linked to Alzheimer's Disease. Several ELISAs targeting Aβ have been developed; however, these ELISAs are known to cross-react with Amyloid Beta precursor protein (APP) and are poorly characterized against monomeric and oligomeric forms of the peptide. The Biosensis MOAB-2 antibody, developed by LaDu and co-workers (Youmans K. *et al.*, 2012), has been shown to specifically detect Aβ, but not the precursor molecule APP. When utilized in ELISAs, the oligomeric form of Aβ peptide (o-Aβ) can be assayed independently of the other forms of the molecule when assayed with the MOAB-2 monoclonal antibody.

The Biosensis oligomeric Aβ ELISA kit is a sandwich ELISA that allows the preferential quantification of oligomeric Aβ peptides. This kit consists of a pre-coated mouse monoclonal anti-Aβ capture antibody (MOAB-2), a biotinylated MOAB-2 detection antibody and horseradish peroxidase (HRP)-conjugated streptavidin. The addition of a substrate (3,3',5,5'-tetramethylbenzidine, TMB) yields a colored reaction product which is directly proportional to the concentration of o-Aβ present in samples and protein standards.

As a result of extensive validation by Biosensis and LaDu's laboratory, this ELISA can be used to accurately determine the level of the oligomeric form of Aβ peptides in tissue extracts and other samples. In addition, the inclusion of a highly validated oligomeric positive control results in a unique ELISA kit for oligomeric detection.

Note: while the concentration of monomeric Aβ peptide used to form the oligomeric complexes is accurately determined, the precise formation, size and number of oligomers cannot be quantified by any known method. We recommend results should be presented as "Aβ peptide equivalents; pg/mL (Arbitrary Units)".

This o-Aβ ELISA kit includes a stabilized, o-Aβ calibrator, a HFIP-treated human Aβ42-peptide standard that has been oligomerized by the treatment described in Youmans K. *et al.* (2012) followed by proprietary stabilization procedures. This o-Aβ standard substitutes the previous HFIP-treated Aβ-peptide which required oligomerization prior to running the ELISA assay.

The purpose of this kit is the *in vitro* measurement of oligomeric Aβ complexes in brain extracts and CSF samples from both transgenic mice and humans relative to a known o-Aβ standard. This kit has not been tested for other sample applications.

3. Materials Provided and Storage Conditions

Reagent	Quantity	
	1 Plate Kit	2 Plate Kit
Capture antibody coated 96 well microplate	12 x 8 wells	24 x 8 wells
Assay diluent B (1x)	2 x 25 mL	4 x 25 mL
Pre-formed, oligomerized Aβ42 standard	2 x 10 ng	4 x 10 ng
Oligomerized Aβ42 positive control (Lyophilized, see vial label for amount)	2 vials	4 vials
Aβ detection antibody (100x)	1 x 110 µL	2 x 110 µL
Streptavidin-HRP (100x)	1 x 110 µL	2 x 110 µL
Wash buffer (10x)	1 x 33 mL	2 x 33 mL
TMB substrate (1x)	1 x 11 mL	2 x 11 mL
TMB stop solution (1x)	1 x 11 mL	2 x 11 mL
Plate sealer	Supplied	

Reagent	Storage and Stability
ELISA kit as supplied & unopened	12 months at 2-8°C
Reconstituted positive control and standard	Reconstitute on the day of the experiment with Assay Diluent B. Storage of unused control sample or standard is not recommended for greater than 2 days at 2-8°C, and the material should not be frozen for best results
Diluted detection antibody and HRP conjugate (1x)	2 weeks at 2-8°C, do not freeze
Diluted wash buffer (1x)	2 weeks at 2-8°C

Note:

- Do not freeze the streptavidin-HRP conjugate
- Do not use assay diluents from other ELISA kits
- The oligomerization protocol has been developed and published by Professor M.-J. LaDu's (Youmans K. *et al.*, 2012)

4. Equipment Required but Not Supplied

- Single and multi-channel pipettes capable of delivering 1 -1000 µL volumes
- Plastic and glassware for sample collection, sample preparation and buffer preparation
- Plate shaker
- Microplate reader capable of reading absorbance at 450 nm

5. Before You Start….

- Read the entire protocol before starting
- Centrifuge all kit components to collect reagents to the bottom of tubes before use
- Bring the microplate and all reagents and solutions to room temperature before starting the assay
- Remove only the required amount of TMB substrate from the fridge to prevent multiple warm-ups and cool downs; keep the TMB substrate in the dark
- Remove the number of strips required and return unused strips to the pack and reseal
- The stop solution provided with this kit is an acid; wear protective equipment when handling
- Please visit our Technical Notes section at www.biosensis.com for further information and helpful hints on ELISA-related topics

Careful experimental planning is required to determine the total number of samples that can be assayed per plate. Refer to Appendix A for help in determining the number of microplates required to conduct an ELISA assay experiment.

6. Sample Preparation

For unknown concentrations of o-Aβ in samples, it is important to perform several dilutions of the sample to allow the o-Aβ concentration to fall within the range of the o-Aβ standard curve (0.03 - 2 ng/mL).

Brain Tissue Extracts & CSF

Extraction of o-Aβ complexes from tissue is straightforward using simple TBS extraction protocols. A suggested sample preparation method for obtaining oligomeric complexes from transgenic mouse brains is provided below. The method has been adapted from that published by Liu et al 2014, [*The Journal of Neuroscience*, 34(39):12982–12999] which closely follows the methods outlined by the LaDu group at University of Illinois at Chicago (see references and methods outlined in Youmans *et al.* (2011 & 2012) for EFAD samples and Tai *et al.* (2013) for human tissue extracts and CSF samples).

In summary, frozen brain hemispheres are bounce-homogenized in TBS containing a complete protease inhibitor mixture and centrifuged at 16,000 x *g* for 30 min at 4°C. The supernatant (TBS-soluble fraction) is collected and stored at -80°C for other use. The pellets are re-suspended in TBS plus 1% Triton X-100 (TBS-T, with protease inhibitors), sonicated for 5 min in a 4°C water bath, and centrifuged at 16,000 x *g* for another 30 min at 4°C. This supernatant is collected and stored at -80°C, and labeled as the TBS-T-soluble fraction, and this fraction should be used in the oligomeric ELISA assay as the sample source. The total protein concentration of all samples should be measured with a detergent (Triton X-100) compatible protein assay. ELISA results then are normalized on the basis of the sample's protein concentration.

7. Preparation of o-Aβ Standard

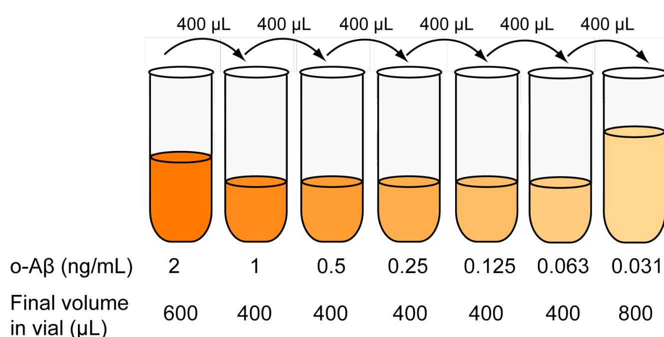
In contrast to previous versions of this kit, the o-Aβ standard is now supplied pre-formed, stabilized and lyophilized as 10 ng/vial of monomeric Aβ equivalents. Reconstitute the standard on the day of the ELISA assay.

Reconstitute 1 vial of standard with 1 mL of Assay Diluent B, vortex and let stand at room temperature for 10 minutes. The initial o-Aβ concentration is 10 ng/mL, label the vial accordingly. Note that the lyophilized material may appear wet which is due to the buffer ingredients and does not affect product quality.

A standard curve is then prepared starting from 2 ng/mL of monomeric Aβ equivalents for the top concentration, followed by a 1:2 serial dilution down to 0.0313 ng/mL. The volumes used for the dilution series depends on the number of repeats per o-Aβ peptide standard. For triplicate measurement (100 µL per well) of each o-Aβ standard concentration, you may want to follow this procedure:

1. Label 7 tubes with 2 ng/mL, 1 ng/mL, 0.5 ng/mL, 0.25 ng/ml, 0.125 ng/ml, 0.0625 ng/ml and 0.0313 ng/ml, respectively
2. Aliquot 400 µL of the assay diluent into each tube except the tube labeled "2 ng/mL"
3. Dilute the 10 ng/mL o-Aβ stock solution 5-fold (200 µL of o-Aβ stock solution added to 800 µL assay diluent); the concentration of o-Aβ is 2 ng/mL
4. Take 400 µL from the "2 ng/mL" tube and transfer to the tube labeled as "1 ng/mL", mix thoroughly
5. Repeat step 4 for each consecutive concentration until the last tube "0.0313 ng/mL" is prepared

Note: Pipet up- and down and vortex to mix; to avoid foaming, use only a very brief vortex.



8. Preparation of o-Aβ Positive Control

Pre-formed oligomeric Aβ has been included in this kit to validate successful assay procedure.

- Reconstitute the lyophilized vial with the volume of Assay Diluent B as indicated on the vial label
- Vortex and let stand for 15 minutes

The expected OD for this positive control is ≥ 1.0 after background subtraction for a TMB incubation time of 5-8 minutes.

9. Other Reagents and Buffer Preparation

- Detection antibody (100x) and streptavidin-HRP conjugate (100x): dilute one hundred-fold with Assay Diluent B; prepare enough volume to add 100 µL per well.
- Wash buffer (10x): crystals may form at the bottom of the bottle; equilibrate to room temperature, mix thoroughly and dilute ten-fold with ultrapure water; each 8-well strip requires about 25 mL of 1x wash buffer per assay.

10. Assay Procedure

1. Add 100 µL of diluted o-Aβ standards, samples, blank (assay diluent only) and positive control to the pre-coated microplate wells
2. Seal the plate (eg., with plate sealer or parafilm) and incubate the plate at 2-8°C for 24 hours.
3. Discard the solution inside the wells and perform 5 washes with 1x wash buffer (200 µL per well). See the technical hints section for a detailed description of the washing procedure
4. Add 100 µL of the detection antibody (1x) into each well
5. Seal the plate (eg., with plate sealer or parafilm) and incubate the plate on a shaker (140 rpm; 0.351 G*) for 60 minutes at room temperature (20-25°C, 70-75°F)
6. Discard the solution inside the wells and wash as described in step 3
7. Add 100 µL of the 1x streptavidin-HRP conjugate into each well
8. Seal the plate (eg., with plate sealer supplied or parafilm) and incubate the plate on a shaker (140 rpm; 0.351 G*) for 30 minutes at room temperature (20-25°C, 70-75°F)

9. Discard the solution inside the wells and wash as described in step 3
10. Add 100 µL of TMB solution into each well and incubate plate at room temperature for 5-8 minutes without shaking in the dark
11. Stop the reaction by adding 100 µL of the stop solution into each well. Visible blue color will change to yellow. Read the absorbance at 450 nm on a plate reader. **Note:** Color will fade over time; hence we recommend plate be read within 5 minutes after adding the stop solution or no longer than 30 minutes after addition. (NOTE: See section 10.9 below)
12. See Appendix C for a troubleshooting guide when unexpected difficulties are encountered

* RCF= 1.12 x Orbit Radius x $(rpm/1000)^2$

11. Technical Hints

1. Do not perform dilutions within the wells of the antibody-coated microplate provided
2. At least duplicate measurements for each standard and sample dilution is recommended
3. Dilute samples to an o-Aβ concentration that falls within the range of the standard curve. Do not extrapolate absorbance readings
4. Avoid touching the inside surface of the wells with the pipette tip
5. Proper emptying and washing the plate is crucial for low background and non-specific binding. For manual plate washing, we recommend the following procedure:
    a. To remove liquid from the wells, place the plate on the palm of the hand and quickly invert the plate over the sink. Forcefully move the arm downwards and stop abruptly to force the liquid out of the wells. When done correctly, this technique should prevent liquid from getting onto the fingers or onto the outside of the microplate wells
    b. Blot and forcefully tap the microplate against clean paper towels for 3-5 times
    c. Wash the wells by pipetting 200 µL of wash buffer into each well and empty the wells as described in step a-b)
    d. Repeat this procedure for a total of 5 times
6. Complete removal of liquid from the wells at each step is essential for reliable results. However, avoid letting the wells dry out at any time
7. Add TMB and the stop solution to the wells in the same order
8. Ensure that all bubbles are removed and that the bottom of the plate is optically clean prior to taking the absorbance reading
9. Stopping the TMB reaction after 5 - 8 minutes is usually sufficient to obtain a very sensitive standard curve. However, TMB incubation times may vary considerable based on the accuracy of diluting the ELISA kit reagents and differences in incubation time and environmental temperature. We use a plate shaker set to 140 rpm in our laboratories when we perform incubations at room temperature (20-25˚C, 70-75˚F). Importantly, we read our plates on a microplate reader capable of measuring up to an optical density (OD) of 4. You may need to adjust the TMB incubation time if your microplate reader has a more limited range.
10. In order to determine the optimal time to stop the TMB incubation, blue color development can be monitored at **650 nm**. Addition of Stop Solution will convert blue into yellow color, with an ~2.5-3X increase in OD at **450 nm**. Once the highest standard reaches an OD of ~1.0-1.4 at 650 nm, immediately stop the reaction by adding Stop Solution. Note that before taking a 650 nm measurement, the plate should be briefly shaken by hand to homogenise the blue TMB reaction product within the wells for most accurate results.

12. Calculation of Results

1. Average the readings for each o-Aβ standard concentration, blank and sample
2. Plot a standard curve with the o-Aβ standard concentration on the x-axis and the OD at 450 nm on the y-axis
3. If values for the o-Aβ standards are adjusted for background absorbance, then subtract the blank value from the OD_{450} of the samples as well
4. Determine the OD_{450} for the positive control sample. An OD ≥ 1.0 after background subtraction for a TMB incubation time of 5-8 minutes indicates acceptable assay performance.
5. Use appropriate software to reduce the data and generate a four parameter logistic (4-PL) curve-fit to assess o-Aβ concentrations in samples; **avoid using linear regression analysis**
6. To calculate the concentration of o-Aβ in the samples read directly from the standard curve or

perform regression analysis. Multiply the result by the sample dilution factor.

Manual Plate Reading

The relative OD_{450} = (the OD_{450} of each well) – (the OD_{450} of Zero well).

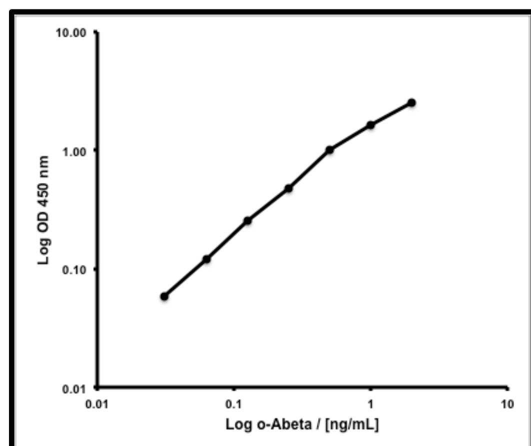
1. The **standard curve** can be plotted as the relative OD_{450} of each o-Aβ standard solution (Y-axis) vs. the respective known concentration of the o-Aβ standard solution (X-axis). The standard curve should result in a graph that shows a direct relationship between target protein concentrations and the corresponding $OD_{450.}$ The greater the concentration of target protein in the solution, the higher the OD_{450}
2. **Determine unknown concentration of o-Aβ in your sample.** The o-Aβ concentration of the samples can be interpolated from the standard curve. Draw a horizontal line to intersect with the standard curve. A vertical line dropped from this point intersects the X-axis at the concentration of o-Aβ in the sample. If the samples measured were diluted, multiply the concentrations from interpolation (see step 1) with the dilution factor to obtain the actual o-Aβ concentration in the sample

13. Typical Data

Standard Curve

Standard curves are provided for demonstration only. A standard curve has to be generated for each o-Aβ ELISA assay.

In addition, we recommend measuring common samples when performing multiple assays across several days in order to normalize the standard curve numbers between the various runs.



o-Aβ / [ng/mL]	OD 450 nm (Background Subtracted)
2	2.52
1	1.63
0.5	1.01
0.25	0.47
0.125	0.26
0.0625	0.12
0.0313	0.06
Blank	0 (0.26)

Dependent on the efficiency of the washing method, the blank OD at 450 nm typically varies between 0.15-0.40.

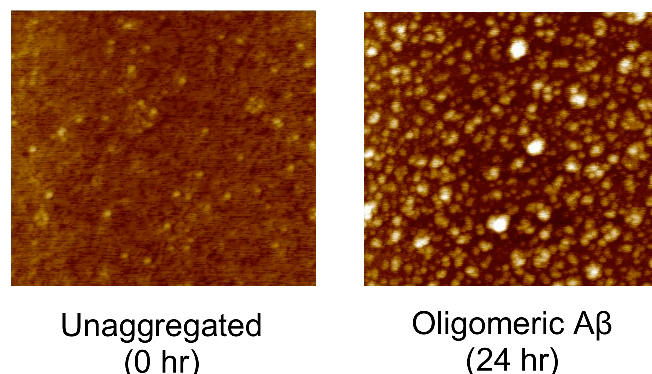
WHY ARE THE RESULTS PRESENTED IN ARBITRARY UNITS? It is important to understand that the number, size and reactivity of oligomers present in either the standard or sample cannot be accurately determined, even with the use of Atomic Force Microscopy or other particle size analysers. Neither can the complexes be reproducibly prepared. Therefore, while the exact amount of Aβ monomers used to produce the o-Aβ standard is accurately known, the final form and concentration of oligomers cannot be determined. We recommend results be presented as equivalents of o-Aβ peptide (ng/mL); Arbitrary Units.

Specificity

The Biosensis o-Aβ Elisa detects Aβ oligomers as validated and described by Youmans *et al.* (2012).

AFM Results on Oligomerized Aβ

The protocol to prepare the oligomeric Aβ standard has been developed in Professor M.-J. LaDu's laboratory. Atomic Force Microscopy (AFM) demonstrates the oligomeric form of the Biosensis standard o-Aβ (Stine *et al.*, 2011).



Unaggregated (0 hr)   Oligomeric Aβ (24 hr)

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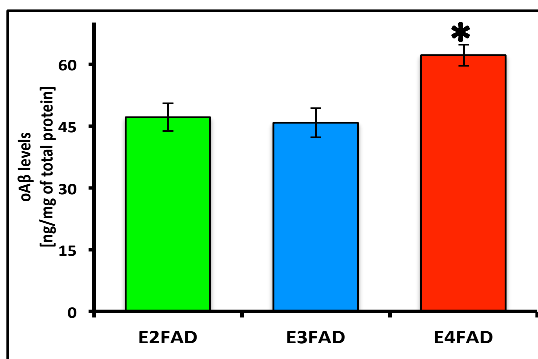
Quantification of o-Aβ in Transgenic Mouse (EFAD) Brain Homogenates

FAD mice were crossed with apoE-targeted replacement mice to produce EFAD mice. o-Aβ levels were measured in TBSX/soluble homogenates (1:4 dilution) from the cortex of 6-month old E2-, E3- and E4FAD mice (n=4) with the Biosensis o-Aβ ELISA kit.



*p <0.05, E4FAD vs. E3FAD and E2FAD. Mean +/- SEM.

This and other experiments have been performed to validate the o-Aβ ELISA kit. For more data and applications of the o-Aβ ELISA kit, please refer to Tai *et al.* (2013).

14. Informational References

Youmans KL *et al.* (2011), **Amyloid-β42 Alters Apolipoprotein E Solubility in Brains of Mice with Five Familial AD Mutations.** Journal of Neuroscience Methods, 196:51-59.

Stine WB *et al.* (2011)**, Preparing Synthetic Aβ in Different Aggregation States**. Methods in Molecular Biology, Volume 670, pp. 13-32.

Youmans KL *et al.* (2012)*,* **Intraneuronal Aβ detection in 5xFAD mice by a new Aβ-specific antibody.** Molecular Neurodegeneration, March 7:8.

Tai ML *et al.* (2013), **Levels of Soluble Apolipoprotein E/Amyloid-β (Aβ) Complex Are Reduced and Oligomeric Aβ Increased with APOE4 and Alzheimer Disease in a Transgenic Mouse Model and Human Samples.** The Journal of Biological Chemistry, 288 (8), pp. 5914-5926.

15. Specific Product Citations

Please see BEK-2215-1P or BEK-2215-2P product listing on www.biosensis.com for the latest product specific publications successfully using our oligomeric Amyloid-β ELISA assays for research applications.

16. Other Information

The Apolipoprotein E/beta-Amyloid (ApoE/A beta) Complex ELISA Kit (cat# BEK-2224) which is based on the MOAB-2 antibody (cat# M-1586-100) and all of our neurodegeneration and disease reagents can be purchased separately from Biosensis at www.biosensis.com.

Appendix A: Determining the Number of Microplate Wells Needed for ELISA Experiments

Standard curve, blank and controls:

- Standard (2 ng/mL, 1 ng/mL, 0.5 ng/mL, 0.25 ng/mL, 0.125 ng/mL, 0.063 ng/mL, 0.31 ng/mL): 7 wells
- Blank (0 ng/mL): 1 well
- Control vial (either provided in the ELISA kit or the researcher): 1 well

Gives a total of 9 wells used per Standard/Control set.

We recommend that the standard curve, blanks, and controls be run on each separate plate for the most accurate calculations. Note that if two (or more) independent assays are run on one plate, each assay requires its own Standard/Control set. We also recommend that ALL samples (i.e. standards, controls, blanks and test samples) be performed in duplicate at least.

Thus, for standards, blanks and controls, 9 x 2 = **18 standard wells are required per assay.** This leaves 96 wells – 18 wells = **78 sample wells per plate** for test samples for EACH of our standard 1P ELISA kits run with a standard curve and controls.

1P kit: 78 experimental wells per 96 well ELISA plate experiment
2P kit: 156 experimental wells per 192 well ELISA plate experiment

The 2-Plate Optional Single Control Set:
Some researchers using our 2P kits run a single, duplicate standard curve, blank and control sample set (18 wells) for the entire 2-plate experiment. This allows 78 test samples on the “standard curve plate” (the plate containing the standard curve, controls and blanks) and a full 96 test samples on the “test only plate” for a total available number of 174 tests per 2 plate kit. (78 wells +96 wells = 174).

2P kit, single set of controls (in duplicate): 174 experimental wells per 192 well ELISA plate experiment

Example: 60 test samples, duplicate measurement at one single dilution
How many wells and plates do I need for sixty test samples at one dilution with a single sample taken before and after treatment?

1. Calculate the number of test samples: 60 samples, x 2 draws each (e.g. before and after) = 120 stock samples
2. Factor in the number of repeat tests desired, in order to calculate the number of wells required. Biosensis recommends that ALL samples be run in duplicate at least.
3. Multiply the number of stock samples by the number of dilutions per sample. In this example, we will be testing EACH stock sample at a single dilution (i.e. 1:100) in duplicate, so 120 stock samples x 2 wells = 240 microplate wells required for sixty test samples. In summary: 60 samples, 4 tests per sample, equals 240 total number of wells required.
4. Decide on the number of standards, blanks and controls run on each plate. We recommend standard, control and blank duplicates for each 96-well plate. Alternatively, one standard, blank and control duplicate set can be run on every 2 plates (2-Plate Optional Single Control Set option discussed above).

Running the Test:
Running the 60 test sample experiment with a standard curve, blanks and controls on *each* test plate, in duplicate (our recommended option):

Single Dilution per Test Sample:

(240 wells required) / (78 wells per plate available) = 3.077 plates required, or just over 3 plates, thus researcher will need to order **4 x 1-plate kits or 2 x 2-plate kits** (there will be unused wells) to ensure enough wells for the entire sixty test samples, tested in duplicate (two draws per sample, 1 dilution, 4 wells per test, total of 240 wells). The unused 8-well strips can be used for other assays later.

Two Dilutions per Test Sample:

If testing at two dilutions, (e.g. 1:50 & 1:100 per sample) and doing duplicates, then the number of wells/tests is doubled to 480 (e.g. 120 x 2 @ 1:50 = 240, PLUS 120 x 2 @1:100 = 480). Then the number of plates is determined by (480 wells required) / (78 wells per plate available) = 6.15 plates required, or just over 6 plates, thus the researcher will need to order **3 x 2-plate kits and 1 x 1-plate kit** to ensure the minimum number of wells for sixty test samples tested in duplicate at two dilutions (two draws per test, 2 dilutions, 8 tests/wells per test, total of 480 tests/wells).

2-Plate Optional Single Control Set Option:

If running 60 test samples with a single dilution, and a single duplicate standard curve, blank, and control sample for every two plates, then one has **174 available test wells per 2P kit**.

For the single dilution, sixty test samples, 2 draws per sample experiment (240 tests), one would need (240/174) = 1.38 2-plate kits, or **2 x 2-plate kits** would need to be ordered to ensure enough wells for all sixty test samples.

For the two dilutions per sample, sixty test samples, 2 draws per sample experiment (480 tests), one would need (480/174) = 2.76 2-plate kits, or **3 x 2-plate kits** would need to be ordered to ensure enough wells for all sixty test samples.

Appendix B: Troubleshooting Guide

This o-Aβ ELISA kit has been developed to deliver reproducible results when following the provided assay protocol. Please refer to the troubleshooting guide below when unexpected difficulties are encountered. If you require further assistance, talk to a scientist at Biosensis (biospeak@biosensis.com).

Problem	Cause	Solution
High background (blank OD > 0.70)	Insufficient washing	Make sure to perform 5 washes after each incubation step with sample, detection antibody and streptavidin-HRP conjugate. Follow the recommended manual washing procedure for optimal washing performance
	Excessive concentration of detection antibody and/or HRP-conjugate	Re-examine the calculations performed to dilute the concentrated stock solutions. Verify that the pipettes dispense the correct volume.
	Increased incubation time and temperature	Please follow incubation times as stated in the protocol and perform incubations at room temperature
	Contamination	Ensure that all plastic- and glassware used is clean. Do not use the same pipette tip to transfer samples and blank solution into the wells
Low absorbance readings	Concentration of o-Aβ in the sample is lower than the detection limit of this assay	Confirm that the assay protocol was carried out correctly by either spiking of o-Aβ into your sample and/or use a sample that is known to contain o-Aβ as another positive control
	Insufficient antibody or insufficient HRP-conjugate	Re-examine the calculations performed to dilute the concentrated stock solutions. Verify that the pipettes dispense the correct volume
	Reagents expired	Ensure correct storage of the kit and do not use the kit beyond its expiry date

Problem	Cause	Solution
Low absorbance readings	Decreased incubation times and temperature	Please follow incubation times as stated in the protocol and warm reagents to room temperature before use
	Microplate wells dried out	Do not leave microplate wells without solution for an extended time
	Wash buffer not diluted	Ensure that the wash buffer is diluted with ultrapure water to a 1x working solution
	Stop solution not added	Add the stop solution to obtain a yellow reaction product that can be measured at 450 nm
Standard OD values above plate reader limit	Excessive incubation with TMB substrate solution	Reduce incubation time by stopping the reaction at an earlier time-point
Sample OD values above standard curve range	o-Aβ concentration in sample is too high	Perform a serial dilution of your sample to obtain a reading that falls within the standard curve
High coefficient of variations (CV)	TMB and stopping solution not added consistently	Add TMB and stopping solution to the wells in the same order and speed
	Inconsistent pipetting	Ensure pipettes are working correctly and are calibrated; review your pipetting technique; add standards and samples to the wells using a single-channel rather than a multi-channel pipette
	Insufficient mixing of reagents	Briefly vortex to mix solutions before pipetting into the wells
	Bottom of the plate is dirty	Clean the bottom of the plate before reading the plate