



Protocol Booklet

Product Code(s)	HB9805, HB8758, HB8524, HB9159
Product Name	β -Amyloid Peptide (1-42) (human) β -Amyloid Peptide (1-40) (human) β -Amyloid Peptide (25-35) (human) β -Amyloid Peptide (42-1) (human)
Purpose	Handling and Aggregating Amyloid Beta

Please note: This product is for RESEARCH USE ONLY and is not intended for therapeutic or diagnostic use. Not for human or veterinary use



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1. Background

Amyloid Beta ($A\beta$) is a family of peptides that form the main constituent of senile plaques in Alzheimer's disease and has been extensively investigated for its role in the aetiology of neurodegenerative diseases. $A\beta$ is produced from the proteolytic cleavage of amyloid precursor protein (APP), a membrane glycoprotein found extensively in synapses throughout the brain (see Figure 1) to result in a family of peptides ranging in size from 37 to 49 amino acids long with the main species being $A\beta$ 1-40 and $A\beta$ 1-42.

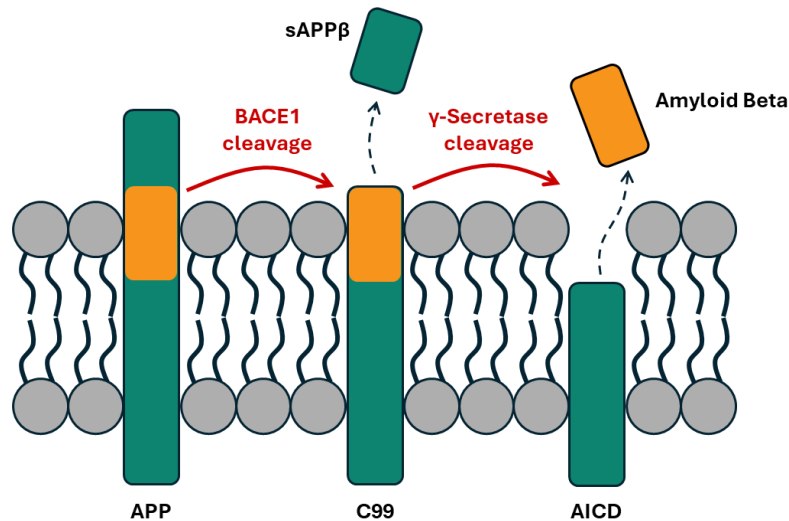


Figure 1. Generation of amyloid beta through APP proteolytic cleavage. Amyloid precursor protein (APP) is cleaved by β -secretases such as BACE1 to give a 99 residue C-terminal fragment (C99) which is then cleaved again by the γ -secretase complex to liberate free amyloid beta which mostly takes the form of either $A\beta$ 1-40 or $A\beta$ 1-42.

Monomeric $A\beta$ has multiple roles in normal physiology where it is involved in trafficking of synaptic vesicles, regulating the excitation / inhibition balance at synapses and activating pathways regulating BDNF release. However $A\beta$ isoforms are prone to aggregation where they form larger and larger complexes and aggregates which are the main driver of their toxicity. $A\beta$ monomers start to coalesce into small soluble oligomers (complexes of 2-20 monomers) which are now believed to be the major toxic species and can diffuse throughout the brain. $A\beta$ oligomers have an array of toxic effects including disruption of synaptic plasticity, promotion of tau hyperphosphorylation and triggering of oxidative stress. Oligomers can coalesce into soluble protofibrils which have additional toxic effects such as being endocytosed by glial cells and being associated with inflammation. These protofibrils can then further aggregate into insoluble fibrils which have a characteristic cross- β sheet pattern which then further accumulate into the plaque deposits found in the brains of patients with Alzheimer's disease.

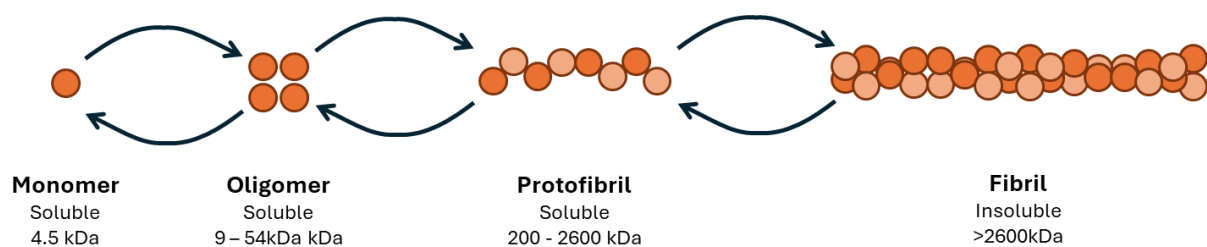


Figure 2. Aggregation of soluble $A\beta$ monomers into insoluble fibrils is a dynamic and concentration dependent process in equilibrium.



There are a range of different A β species but the most widely studied in neurodegenerative research are:

- Amyloid β 1-40 - comprises about 90% of the total A β formed in the brain and while more soluble than A β 1-42 still forms a major constituent of senile plaques in Alzheimer's disease.
 - Available from Hello Bio as [HB8758 - \$\beta\$ -Amyloid Peptide \(1-40\) \(human\)](#), a high purity lyophilized monomer in a 1mg pack size.
- Amyloid β 1-42 - comprises only around 10% of A β in the brain, however it is less soluble, more neurotoxic and quicker to aggregate than A β 1-40 due to a higher propensity to form β -sheet structures.
 - Available from Hello Bio as [HB9805 - \$\beta\$ -Amyloid Peptide \(1-42\) \(human\)](#), a high purity lyophilized monomer in packs ranging from 100 μ g to 1mg.
- Amyloid β 25-35 - produced from the cleavage of A β 1-40 and is another component of senile amyloid plaques in the brain. A β 25-35 is also able to aggregate into fibrils and has been found to have cytotoxic effects.
 - Available from Hello Bio as [HB8524 - \$\beta\$ -Amyloid Peptide \(25-35\) \(human\)](#), a high purity lyophilized monomer in a 1mg pack size.
- Amyloid β 42-1 - a reverse form of the A β 1-42 peptide that is used as a control peptide in experiments using A β 1-42 as, while it has been found to aggregate, it is much less toxic to cells than A β 1-42
 - Available from Hello Bio as [HB9159 - \$\beta\$ -Amyloid Peptide \(42-1\) \(human\)](#), a high purity lyophilized monomer in a 1mg pack size.

2. Handling Monomers

Amyloid β monomers supplied by Hello Bio are provided as a lyophilizate that has been previously treated with HFIP. We recommend to dissolve peptides following the below NH₄OH protocol although others are provided in section 3.1.

- 1) Dissolve A β peptide in 1% ammonium hydroxide (NH₄OH) using a volume of around 70-80 μ l per 1mg of peptide. Briefly spin the vial at >1000g to bring all the solution to the bottom of the vial.
 - a. 1% ammonium hydroxide is conveniently available preformulated from Hello Bio as [HB9790 - 1% NH₄OH solution for dissolving Beta-Amyloid peptides](#)
 - b. Do not store the peptide in 1% NH₄OH
- 2) Immediately dilute the A β solution in ice-cold PBS to a concentration of $\leq$ 1mg/ml. Pipette up and down to ensure the solution is well mixed.
- 3) Either use immediately or snap-freeze the solution in aliquots and store at -80°C.
 - a. Before use it is recommended to spin the A β aliquot at full speed in a benchtop centrifuge to remove any small insoluble aggregates that have formed.

We recommend following these tips to avoid undesired aggregation of A β peptides:

- Use clean tools to avoid introducing contaminants into the solution that could be seeds for aggregation.
- Avoid extensively vortexing A β samples as this can promote aggregation.
- Be careful to avoid introducing bubbles into A β solutions as this can be another source of seeds for aggregation.
- Avoid freeze-thaw of A β solutions as this has been found to promote aggregation.

3. Aggregation of Amyloid Beta

There are a diverse range of methods used to generate different oligomerisation species of A β each with their own range of advantages and disadvantages. We have a protocol based upon the work of [Stine et al., 2011](#). It is important to start with a solution of pure A β monomers to maximize the probability of resulting in a uniform aggregation state at the end.

3.1 Starting with Pure Monomeric A β

There are a few different methods that are used to ensure a solution of pure monomeric A β . **Please note: if planning on oligomerizing A β then we recommend dissolving straight from the vial with DMSO (please see section 3.3).**

HFIP Treatment

1. Dissolve the A β peptide in HFIP (1,1,1,3,3,3-hexafluoro-2-propanol, CAS: 920-66-1) and vortex briefly to mix.
2. Dry the solution under a stream of nitrogen
3. Redissolve the peptide in HFIP at 1mg/ml, sonicate in a bath sonicator for 5 minutes.
4. Dry the solution under a stream of nitrogen
5. Redissolve the peptide in HFIP at 1mg/ml, sonicate in a bath sonicator for 5 minutes.
6. Dry the solution under a stream of nitrogen
7. Redissolve the peptide in HFIP at 1mg/ml, aliquot into smaller volumes appropriate for a single experiment then sonicate in a bath sonicator for 5 minutes.
8. Dry the solution under a stream of nitrogen followed by drying under vacuum for 1-2 hours until the peptide is completely dry.
9. Store the A β aliquots at -80°C until further use.

NaOH Treatment

1. Dissolve the A β peptide in 10mM NaOH to 1mg/ml and sonicate for 30 minutes using a sonicator bath.
2. Aliquot and snap freeze before storing at -80°C
3. When ready to use, thaw rapidly at 37°C then remember to account for the high pH of the A β solution when using it.

NH₄OH Treatment

1. Dissolve A β peptide in 1% ammonium hydroxide (NH₄OH) using a volume of around 70-80 μ l per 1mg of peptide. Briefly spin the vial at >1000g to bring all the solution to the bottom of the vial.
 - a. 1% ammonium hydroxide is conveniently available preformulated from Hello Bio as [HB9790 - 1% NH₄OH solution for dissolving Beta-Amyloid peptides](#)
 - b. Do not store the peptide in 1% NH₄OH
2. Immediately dilute the A β solution in ice-cold PBS to a concentration of $\leq$ 1mg/ml. Pipette up and down to ensure the solution is well mixed.
3. Either use immediately or snap-freeze the solution in aliquots and store at -80°C.
 - a. Before use it is recommended to spin the A β aliquot at full speed in a benchtop centrifuge to remove any small insoluble aggregates that have formed.

3.2 How to Monitor Aggregation

Being able to monitor the progression of Amyloid Beta aggregation is critical to understand what species are being used in experiments where A β is aggregated then used as an *in-vitro* or *in-vivo* manipulation. There are multiple ways of measuring A β aggregation with it being recommended to use more than one method for a more accurate determination of A β aggregation status.

Fluorescent Assays

There are a range of fluorescent molecules that differentially bind to A β fibrils and fluoresce compared to monomers which are available from Hello Bio:

- [HB5134 - Thioflavin S \(ThS\)](#)
- [HB17774 - Thioflavin X \(ThX\)](#)
- [HB7143 - Thioflavin T \(ThT\)](#)
- [HB5252 - Methoxy-X04](#)
- [HB0737 - Congo Red](#)

Of these dyes Thioflavin X and Thioflavin T are suitable for quantitative measurement of A β aggregation with Thioflavin X being approximately 5x brighter with 7x higher affinity to A β compared to Thioflavin T.

1. Prepare A β in 20mM phosphate buffer containing 0.2mM EDTA, 1mM NaN₃ and 20 μ M Thioflavin T or 10 μ M Thioflavin X at pH 8. A β concentrations of over 1 μ M exhibit aggregation within a 24 hour timespan.
2. Incubate for 24 hours at 37°C regularly measuring fluorescence at either:
 - a. Thioflavin X: Excitation: 420, Emission: 494nm
 - b. Thioflavin T: Excitation: 450nm, Emission: 485nm

Transmission Electron Microscopy (TEM)

A β fibrils are large enough to be visualized using transmission electron microscopy (TEM) with uranyl acetate counterstaining. A standard protocol from [Xiao et al., 2015](#) is:

1. Load 10 μ l of a fibril sample onto a 300 mesh copper formvar / carbon grid and incubate for 1 minute before blotting off any excess.
2. Counterstain the sample with 10 μ l of 2% uranyl acetate for 90 seconds before blotting off any excess and drying the grid in a desiccator chamber.
3. Image using a TEM at 80kV and a magnification of approximately 120,000x

Dynamic Light Scattering

Dynamic Light Scattering (DLS) is a technique that uses the scattering of a laser beam off small particles in solution to work out their size distribution (for an example see [Jeon et al., 2023](#)). This can be applied to monitoring A β aggregation as DLS is able to non-invasively measure A β aggregation in a timecourse and distinguish between small soluble oligomers and larger insoluble fibrils.

Atomic Force Microscopy (AFM)

Atomic Force Microscopy (AFM) is a technique where a nanoscale probe is dragged across a sample then the deflection measured to map the surface topology of a sample. This has been successfully applied to monitor the aggregation of A β protofibrils into fibrils (see [Harper et al., 1997](#)) and can directly visualize the structure of aggregated A β and even has the resolution to visualize the helical structure of mature fibrils.

3.3 Aggregation Protocols

Depending on the size of aggregate you require for your experiments there are different protocols which bias their conditions towards making fibrils assemble to a certain range of sizes. We recommend in all cases dissolving in DMSO straight from the vial for aggregating A β .

3.3.1 Preparing Oligomers

1. Dissolve monomeric A β in DMSO to a 5mM concentration at room temperature (22.6mg/ml for A β 1-42)
2. Dilute the A β down to 100 μ M (0.45mg/ml for A β 1-42) using ice-cold F-12 cell culture media (phenol free and containing 146mg/L L-glutamine)
3. Vortex for 15 seconds then incubate at 4°C for 24 hours.

3.3.2 Preparing Fibrils

1. Dissolve monomeric A β in DMSO to a 5mM concentration at room temperature (22.6mg/ml for A β 1-42)
2. Dilute the A β down to 100 μ M (0.45mg/ml for A β 1-42) using room temperature 10mM HCl.
3. Vortex for 15 seconds then incubate at 37°C for 24 hours.

3.3.3 Preparing Larger Insoluble Aggregates

1. Dissolve monomeric A β in DMSO to a 5mM concentration at room temperature (22.6mg/ml for A β 1-42)
2. Dilute the A β down to 100 μ M (0.45mg/ml for A β 1-42) using room temperature 10mM HCl + 150mM NaCl.
3. Vortex for 15 seconds then incubate at 37°C for 24 hours.

3.4 Separating A β Aggregates

Depending on the experimental requirements, the A β aggregate mixture can be used directly or purified to isolate specific species. There are a few different methods used to separate A β species following an aggregation protocol:

- Filtration is an easy method to remove larger A β fibrils and aggregates with spin-column filters being available with a wide range of molecular weight cut offs (MWCO), ranging from as little as 1kDa up to over 100kDa. Spin filtration is extremely quick, however is relatively crude as it only removes A β species above the MWCO, leaving a mixture of smaller species remaining.
- Size exclusion chromatography (SEC, see [Nichols et al., 2015](#)) is the main method used to separate A β species where A β species are separated by hydrodynamic size as they move through the resin bed with larger species eluting before smaller oligomers and monomers. SEC is an extremely powerful technique however does require expensive columns and machinery to carry out.
- A β species can also be separated using centrifugation (see [Ward et al., 2000](#)). Mixed A β samples can be added to a density gradient and spun at extremely high g-force (approx. 350,000 g_{av}) to separate the A β species by their molecular weight. This does however require expensive specialised centrifuges and long run-times making this method non-trivial.

4. Uses of Amyloid Beta Aggregates

Creation of high-purity and defined aggregation state amyloid beta species is critical to enable research into the specific molecular mechanisms underlying A β toxicity *in-vivo* which leads to Alzheimer's disease. Some of the most common uses for A β oligomeric species include:

- Screening for either small molecule or peptide inhibitors of A β aggregation or disaggregators of A β fibrils using high-throughput screening assays. Dyes such as [Thioflavin X \(ThX\)](#) and [Thioflavin T \(ThT\)](#) increase fluorescence in response to the formation of aggregated A β species therefore can be readily adapted into a high-throughput format.
- *In-vitro* toxicity assays where A β species are added to cells (e.g. primary or iPSC derived) neurons and the toxicological and molecular mechanisms of cell damage are assayed using tests such as H₂DCFDA to measure reactive oxygen species, Annexin V assays to measure apoptosis or MTT to measure cell proliferation.
 - [HB7375 - DCFDA / H2DCFDA - Cellular ROS Assay Kit](#)
 - [HB9623 - Annexin V-FITC Apoptosis Staining / Detection Kit](#)
 - [HB8164 - Annexin V-PE Apoptosis Staining / Detection Kit](#)
 - [HB5283 - MTT](#)
- *In-vivo* models where animals are injected with toxic A β species to study their impact on a systems level. This is often carried out on genetically modified animals that overexpress human A β isoforms which then have A β fibrils injected to trigger further aggregation of host A β which produces an Alzheimer's like phenotype in a greatly shortened timespan compared to the natural disease process. Researchers commonly carry out immunohistochemical analyses to identify the specific pathology on different cell types such as:
 - Neurons (e.g. [Anti- \$\beta\$ III Tubulin antibody ValidAb™](#), [Anti-NeuN antibody ValidAb™](#), [Anti-MAP2 antibody ValidAb™](#))
 - Glia (e.g. [Anti-GFAP antibody ValidAb™](#))
 - Oligodendrocytes (e.g. [Anti-Myelin Basic Protein \(MBP\) Antibody ValidAb™](#))

Fluorescent dyes can also be used to visualize A β plaques in histological samples from animal models such as:

- [HB17774 - Thioflavin X \(ThX\)](#) – next generation dye that is 5x brighter than ThT
- [HB7143 - Thioflavin T \(ThT\)](#)
- [HB5134 - Thioflavin S \(ThS\)](#)
- [HB5252 - Methoxy-X04](#)
- [HB0737 - Congo Red](#)

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