Amyloid-Beta Tech Support FAQs

Last Updated: December 2023
Last Updated by: Christina K.

Q: For an internal *in vitro* test, we would like to run a SH-SY5Y culture (ideally combined with a microglia cell line) and induce/add/recreate B-amyloid aggregates/plaques.
I found on your website that you have B-amyloid oligomers, would these be appropriate for this setup? Are these proteins tested with (preferably SH-SY5Y) a neuronal cell line for toxicity?

A: We have both oligomers and fibrils available as well as HFIP-treated monomeric peptides. We do not yet have pathology data on these products, and have not yet tested in SH-SY5Y cells, but we have shown that the fibrils and oligomers are toxic to primary rat cortical neurons. We also have AFM, TEM, and western blot data using an anti-amyloid beta 6E10 antibody. (**Amyloid beta preps are produced at 1mg/ml**) (03/10/2022)

Q: What QC is performed on the oligomers / fibrils?

A: For the time being, the QC will include (1) Western Blot with anti-amyloid beta 6E10 antibody showing oligomeric or fibrillar species and (2) AFM imaging showing globular oligomers or fibrils. We have shown if these QC parameters pass that they are toxic to primary cortical neurons. We had some issues with ThT signal so we avoided including this. We may add batch ThT and toxicity QC at a later date but for now it's WB and AFM. (Internal use 3/10/2022)

Q: How stable are these and how long have you cultured neurons in the presence of them?

A: Stability-wise, we haven't yet tracked their degradation within cultures. On a benchtop at room temp the PFFs are quite stable through a freeze-thaw and for weeks in HCl yet tend to clump at physiological pH (hence our use of sonication before adding to cells). We know the oligomers are stable through a freeze-thaw and maintain their size throughout size-exclusion chromatography but haven't tested beyond this as literature suggests they may further aggregate. (4/6/2022)

Q: Would you recommend a one-time administration to a neuronal culture or repeated addition? Saw your data on the viability after 14 DIV in rodent neurons, was that after one-time stimulation?

A: While we are in the midst of further studies in different cell lines (and animal models), we have currently only finished studies in primary rat cortical neurons. The cells were cultured at 37°C in a humidified air (95%)/CO2 (5%) atmosphere and half of the medium was changed every 2 days with fresh medium. The AB preparations were added once after 7 days of culture and grown for an additional 14 days with regular media changes prior to fixing/IHC/cortical neuron counts. (4/6/2022)

Q: Would you recommend PFFs or oligomers for study using iPSC-derived neurons to look at morphology and firing properties?

A: In terms of recommending PFFs or oligomers, I would try both if feasible, as the literature on these aggregates is quite convoluted and you may see differing effects depending on what you are looking at. I would note that in our studies (and many others) the PFFs are initially sonicated prior to adding to cells, which breaks them up into much smaller, more soluble, oligomer-like structures. If you don't have the capability for recommended sonication (using a Bioruptor Pico), or if only one construct is feasible for the extent of your experiment, I would go with the oligomers as most literature points towards these being the more physiologically active. (4/6/2022)

Q: Do you currently have any morphometric data?

A: We observed potent toxicity (~50%) with 45 ug/mL concentrations of either oligomers or fibrils, which appears to be dose-dependent in primary rat cortical neurons. We didn't see toxicity with any levels of monomer tested. We did not go into morphometric data at that time. (4/6/2022)

Q: For SPR-487 (fibrils), is it soluble (protofibirls) or insoluble fibrils (which can be centrifuged down under 16 kg 5 min)?

A: SPR-487 amyloid-beta fibrils are soluble in the storage buffer we sell them in (10mM HCl 2% DMSO), at a neutral pH these fibrils become insoluble. We recommend sonicating immediately prior to use. (6/16/2022)

Q: For SPR-488 (oligomers), I have seen the AFM picture, but would like to know whether you have run a size-exclusion chromatograph to check its size? Do you know the oligomer estimated molecular weight?

A: We have run SEC on SPR-488, however, we do not typically share lot-specific QC data. The molecular weight of the oligomers varies from ~37-75 kDa. (6/16/2022)

Q: For both oligomers and fibrils, did you completely remove the unaggregated monomers, or it is a mixture of aggregates and monomers? From the WB gel shown on your product page, there is a monomer/dimer band present in both products.

A: These are disordered proteins that exist in equilibrium. Unfortunately, we cannot remove monomers from oligomer and fibril preps because monomers aggregate readily on filters. Thus, there is likely still some monomer present in these preps, however it is difficult to quantify as SDS breaks down aggregates into monomers. The Western blot images you noted on our website shows the monomer, oligomer, and fibril preps run at the same concentration, and there is <5% of the monomer signal left in the oligomer and fibril preps, even in the presence of SDS which breaks aggregates down into monomers. (6/16/2022)

Q: Customer was interested in a protocol for resuspension in an injection friendly buffer, as her colleague had been trying unsuccessfully to reconstitute/dilute in PBS; the protein always aggregates *(I believe she was using another company's abeta monomer, not ours*).

A: Abeta can be tricky – it definitely requires quality peptide and an initial HFIP treatment of the peptide to remove secondary structure and careful resuspension to generate monomer. We sell pre HFIP-treated monomer (SPR-485, https://www.stressmarq.com/products/protein/amyloid-beta-protein-spr-485/?v=3e8d115eb4b3) that can be successfully re-suspended into soluble monomer using DMSO/water.

A few important notes - DMSO must be used from a freshly opened ampule as DMSO rapidly absorbs moisture from the air. Also, cold dH2O must be used and re-suspended monomer should be used for experiments immediately. I've included our protocol for re-suspension of our HFIP-treated peptide below. **This protocol yields 1 mg/mL abeta monomer in a 2% DMSO solution.** This resuspension can then be diluted directly into PBS or media such as F-12 or neurobasal and we haven't seen any adverse effects on primary rat neurons at up to 45 ug/mL (i.e. a >1/20 dilution so a final DMSO concentration <0.1%). We have not yet tried injecting these into mice but I would expect the primary culture to be a good indicator this would be ok. If you are looking to induce abeta specific pathology, however, I would use oligomers or fibrils as opposed to monomers (which we also sell).

1. Bring 500 ug of SPR-485 powder to room temp for 10 minutes, ensure cold filter sterilized (F.S.) Ultra-pure water is available, and pre-cool benchtop centrifuge to 8°C.

2. In BSC, open 5 mL glass vial of Hybri-Max DMSO (Sigma, Cat # D2650-5x5ML). This type of DMSO has less than 0.1% of water. As soon as the DMSO is opened, atmospheric air will contaminate it and increasing the water content. It cannot be stored and re-used for peptide re-suspension.

3. Add 10 µl fresh DMSO to peptide aliquot, mix by pipetting up and down and scraping walls of tube. This should take 2-3 minutes – continue until "beading" on side of tubes is minimized as much as possible.

4. Vortex peptide for 5 seconds and bath sonicate for 5 minutes.

5. Add 490 µl of cold F.S. Ultra-pure water to DMSO dissolved peptide, mix thoroughly by pipetting, vortex 5 seconds.

6. Centrifuge the material at 14,000xg at 8°C for 5 minutes and transfer supernatant to new Axygen® Maxymum Recovery® tube (VWR, Cat # 22234-046).

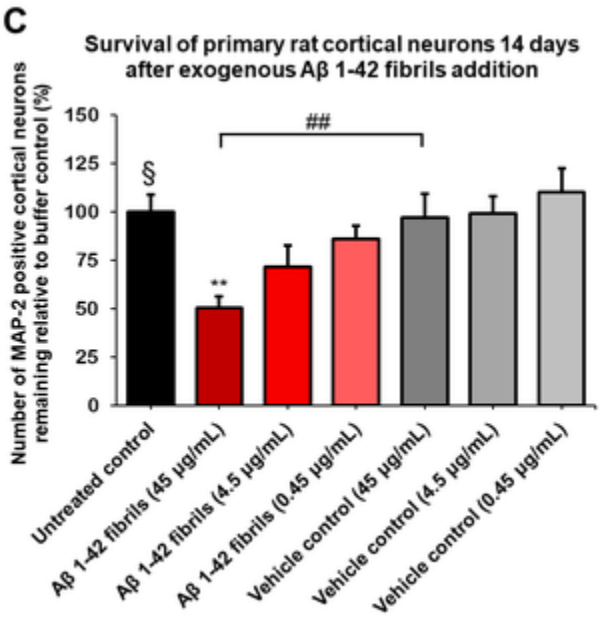
While the centrifugation is in progress, make 2% DMSO in F.S. Ultra-pure water – this will be used as diluting buffer and nanodrop blanking buffer.

7. Check concentration on nanodrop. Dilute the material to 1 mg/mL in 2% DMSO in F.S. Ultra-pure water if necessary. Make sure to blank with 2% DMSO solution.

From there you should be able to immediately dilute in buffer for immediate injection. I hope this helps.

Q: I plan to treat hiPSC-derived neurons with your product SPR-847. I was wondering if you had any recommendations for administering this treatment without confounding cytotoxicity from the storage buffer. Any advice on dosage and/or duration of treatment with this product would be greatly appreciated.

A: The highest concentration we tried on primary neurons was 45 ug/mL, which is essentially a 20-fold dilution in neurobasal media of the fibril stock we sell in that HCl buffer. As you can see the buffer diluted the same way had no effect. At 10 mM HCl you're essentially diluted to 0.5 mM HCl which will be negligible in any properly buffered system (like 20 mM Hepes pH 7.4 for example)



Q: What is the molecular weight since I'd use a molar concentration for the research?

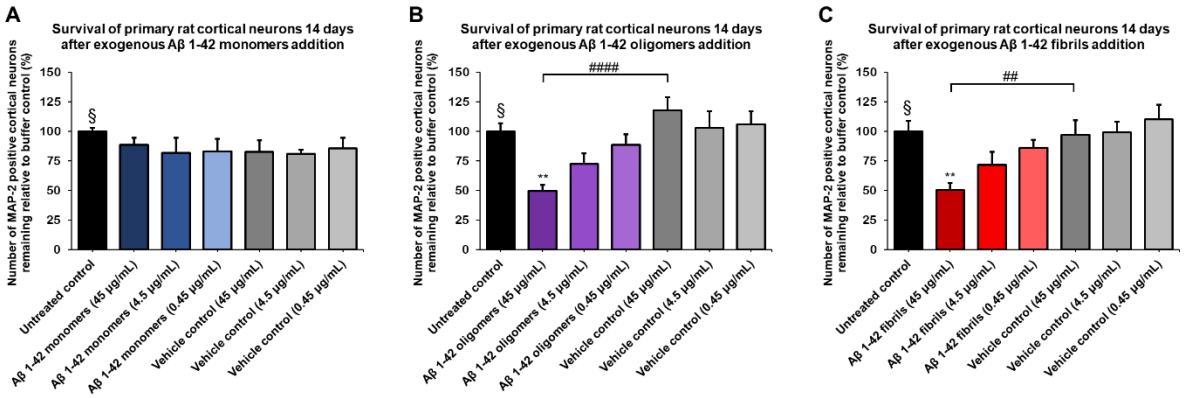
A: The molecular weight of the oligomers varies from ~37-75 kDa.

Q: We recently purchased Abeta 1-42 oligomer and fibrils from you. From the concentration that was labeled on the vial, the oligomer or fibrils are at 1mg/ml. I would like to know what is the mM or uM does 1mg/ml converts to?

A: According to the following formula (µM = (µg/L) / (MW in Da)), and a M.W of approximately 4.5kDa, the molar concentration of our 1mg/mL amyloid beta monomer would be ~222µM. We use the M.W of the monomer as we don't know exactly how many units are in each amyloid beta oligomer/fibril.

Q: Are there any cytotoxicity references of this product?

A: This product was launched earlier in 2022 and therefore we do not yet have any published citations where the product has been used. However, from testing that has been performed with our amyloid beta oligomers, fibrils and monomers, we observed potent toxicity (~50%) with 45 ug/mL concentrations of either oligomers or fibrils, which appears to be dose-dependent in primary rat cortical neurons. We didn't see toxicity with any levels of monomer tested. For your perusal I have attached an image that outlines our testing for toxicity. For your interest and to find additional information, this image can also be seen on the product webpage for amyloid beta oligomers.



Q: I recently bought the amyloid beta protein (human synthetic amyloid beta 1-42 pre-formed fibrils) catalog no. SPR-487 Lot No. XA330551. I had a question about the pH of the storage buffer, 10 mM HCl and 2% DMSO. What pH is the storage buffer? On the tau protein catalog no. SPR-477 Lot No. PL387406 includes that the storage buffer is pH 7.4 and I was just wondering if this is a similar case with the amyloid or if the amyloid is in a more acidic storage buffer? Is there an estimate of how low the pH is? I am just trying to evaluate if it will be an issue for intracerebral injection and if I will need to do anything to it prior if the pH is very low.

A: The amyloid beta fibrils (catalog # SPR-487) are stored in an acidic buffer (10 mM HCl + 2% DMSO) with low pH. The DMSO, low pH and lack of salt are required for proper fibril formation and longer stability.

Please note that the amyloid beta fibrils are soluble in the acidic buffer, however they tend to aggregate when put into neutral buffers and become insoluble. In this case we recommend sonicating immediately prior to use. A pH strip shows pH 2-2.5, however this solution has very little buffer capacity, so it is not very accurate. That said, as it does have a lower buffer capacity, when diluted into buffer or media for experiments it will have minimal impact on final pH. Please keep in mind that due to their aggregation in different buffers you need to sonicate fibrils before injection.

Q: I had ordered the beta amyloid oligomer (SPR-488) which is in the storage buffer Phosphate buffer (PB) pH 7.4 and 10 mM NaCl as mentioned in your catalog. I wanted to use this buffer alone as a control in my experiments. Could you please let me know if the Phosphate Buffer pH7.4 is *Sodium* Phosphate or *Potassium* Phosphate? Also, what is the Molarity of the Phosphate buffer in the below mentioned storage buffer? Is it 100mM?

A: The storage buffer for our amyloid beta oligomers (catalog# SPR-488) is 10 mM Potassium phosphate, 10 mM NaCl.

(Internal Use) **Vicky:** Looking at our recipes, we use 10 mM Potassium phosphate to generate the oligomers. However, after final additions, I believe we end up somewhere around 8 mM PB, 10 mM NaCl in the final product. I am not sure how much of this information would be considered proprietary, since our recipes are technically confidential.

Jacob: Not proprietary to tell them the final buffer composition but is proprietary to explain why it's in that buffer (doesn't work with high salt, requires NaOH reusspension and HCl titration etc). It's basically 80% 1X PB (no proclin) and 10 mM NaCl final. I'd rather not complicate the product explaining that all on the page or info sheet, 99% of people will see phosphate buffer and never ask, and 90% will be diluting into something else to make it irrelevant. They are likely asking because there are a few variations of phosphate buffer depending on KCl/NaCl composition, so just give them the amounts, but you'll have to calculate based on our recipe.

Date: April 26, 2023

Written by: Samantha

Q: What is the concentration or molarity of the phosphate buffer in which the oligomers are stored in? From the datasheet, I know it is at pH 7.4 and has 10 mM NaCl, but I wanted information on the concentration of the phosphate buffer itself so I can match the vehicle control properly.

A: I am happy to help! The storage buffer for our amyloid beta oligomers (catalog# SPR-488) is 10 mM Potassium phosphate, 10 mM NaCl.

Q: Thanks! Can you clarify what type of potassium phosphate? Because there monobasic, dibasic, and tribasic potassium phosphates.

A: This is our 1X PB recipe (for 1.0 L): *(provided by Jacob)*

- 1.361g of KH_2PO_4
- 7.5 mL of 1M NaOH
- Up to 1.0 L with dH_2O
- F.S. and autoclaved prior to use

* For SPR-488, these also have 10 mM NaCl present in addition to the 1X PB.

Q: Is it possible to receive an aliquot of the exact storage buffer used for the product for a vehicle condition? If not, I can make it myself but feel it would be best to use the exact solution. For SPR-487 for example, I see the storage buffer is 10 mM HCl + 2% DMSO. Is the solvent in this case water or PBS/saline solution? To make it myself, I guess I would order a 37% HCl solution (Sigma, 320331) which is 12.178M and dilute it to 10mM by adding 8.2uL to 10mL water, then adding 2% DMSO?

A: (Jacob's response) This is correct, if we assume the molarity of concentrated 37% HCl stock is ~12.178M, their calculation for 10 mM HCl is correct, and we do indeed add 2% v/v DMSO to this (example 20 uL DMSO + 980 uL 10 mM HCl). Just be careful when adding concentrated HCl to water – you don't want to breathe those fumes – and make sure the DMSO is fresh and >98% pure.

Unfortunately, we do not provide the buffer itself. However, the recipe would be correct in that example. Just be careful when adding concentrated HCl to water – you don't want to breathe those fumes. And make sure the DMSO is fresh and >98% pure!

Q: I am interested in using item SPR-487B (Amyloid Beta Pre-Formed Fibrils) in an in-vitro cell assay. Could you please let me know whether the fibrils are sterile and endotoxin tested?

A: All our protein constructs including amyloid beta have passed sterility tests which is also part of our QC. We do not run an endotoxin testing for amyloid beta since it's a synthetic peptide. We have tested them in the past and endotoxin levels were <0.1 EU/mL so, we do need to test every batch we release.

Q: We have recently purchased Abeta1-42 monomers (Cat. No SPR-485B), oligomers (Cat. No SPR-488B) and preformed fibrils (Cat. No SPR-487B) and are currently planning a series of different experiments. In your online catalog, you are showing beautiful product images. I would be particularly interested in learning a few more technical details on the Western blot analysis on the three amyloid preparations (image 2/5). The image looks great, and we would certainly like to include similar Western blots in our series of experiments. In the legend to this product image, I found the following information: "Amyloid beta constructs at 160 pmol were run on 4-12% Bis-Tris SDS-PAGE, transferred to nitrocellulose in the presence of 0.02% v/v Tween-20…". I was wondering if you could send me the complete recipe of the blotting transfer buffer that was used, the information which kind of blotting device was used (tank blot, semi-wet transfer unit or semi dry blot?) and possibly the transfer time and settings. This information would probably help us a lot to save time and money. In case the protocol and images have been published, I would greatly appreciate if you could let me know the reference.

A: WB Analysis on our Amyloid beta products:

SDS-PAGE: we use these Millipore precast Bis-Tris 4-12% gradient gels with their pre-mixed MES buffer following manufacture's protocol. This is very important for resolving 5 kDa monomers and 50 to >250 kDa oligomers/fibrils simultaneously (https://www.sigmaaldrich.com/CA/en/product/mm/mp41g15?gclid=EAIaIQobChMIxYH9g-nsggMVWIjCCB3m_wvAEAAYASAAEgLb5fD_BwE). You need to make sure you have a ladder with at least a 5kDa marker as a control for your transfer. We use the Biorad Dual Xtra ladder which has both a 2 kDa and 5 kDa, up to 250 kDa. *Do not remove the stacking gel. You can truncate a small bit off the sides of*

the wells, but the fibrils mostly remain at the bottom of the wells at the interface with the stacking gel – if you remove these obviously there will be nothing to transfer.

Blotting Transfer: we use the Biorad Tank blot and nitrocellulose membranes. We run 100 volts (constant) for 1 hour. Our transfer buffer is cold, and we add a -20 ice pack to the tank outside of the cassette to keep it that way, although you could just run in a cold room. We've found the 0.02-0.05% Tween-20 is very important for successful transfer of oligomers and fibrils. Make sure you properly overlap the nitrocellulose on the gel so that you are transferring everything from the monomers around 5 kDa to the wells where the fibrils will be. Removing bubbles is also critical. Finally, we do not ponceau stain, we found it doesn't visualize the oligomers/fibrils well – visualization of all MW markers is enough confirmation of successful transfer. We then dry, block with 5% skim milk in TBST, wash, 1:1000 6E10, wash, 1:4000 secondary, wash, detect with ECL.

- ***10X Transfer Buffer (1L):***
  · 30.3 g Tris Base
  · 144 g Glycine
  - Up to 1L with Ultra-pure water, mix, filter sterilize, and store at room temperature (23°C).

- ***1X Transfer Buffer (1L):***
  · 100 mL 10X Transfer Buffer

  · 200 mL Methanol

  · 700 mL Ultra-pure water (4 deg C)
  - Mix fresh, immediately before use. Add 0.02-0.05% Tween from 20% f.s. stock. Do not reuse.

Q: We received an inquiry about Amyloid Beta 1-42 Pre-formed Fibrils (SPR-487). Is this protofibril, or fibril? The customer is looking for the protofibrils.

A: Our Amyloid beta PFFs (SPR-487) are considered fibrils but if we look at the TEM we see a population of longer fibrils and some shorter almost oligomeric ones that may be "protofibrils". We haven't fully characterized their molecular structure to say if there's an actual difference between them. Our amyloid beta fibrils are generated based on the fibrilization methods of this paper by Stine *et al.* https://www.jbc.org/article/S0021-9258(19)32451-2/fulltext.