Product Data Sheet

Product Name: 1% NH_4OH solution

Lot Number: See label on vial

Catalog Number: AS-61322

Volume: 300 µL

Description: This solution can be used as the solvent for the following beta-amyloid 1-42 and 1-40 catalog peptides*:

Beta-amyloid 1-42		HiLyte 488-Beta-Amyloid 1-42
AS-24224 AS-20276 AS-20276-5 AS-20276-25	AS-21791 AS-21793 AS-60883-01 AS-60883	AS-60479-01

Beta-amyloid 1-40		HiLyte 488-Beta-Amyloid 1-40
AS-24236 AS-24235 AS-24236-5	AS-20698 AS-23211	AS-60491-01

Molecular Weight: 35.05

Appearance: Clear liquid

1% NH_4OH Storage: Room temperature

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Peptide Reconstitution:

Add 1% NH_4OH directly to the lyophilized peptide powder using 35-40 µL to 0.5 mg peptide or 70-80 µL per 1 mg peptide. The peptide cannot be stored long term in 1% NH_4OH, and it is therefore important to immediately dilute this solution with 1X PBS or other buffer to a concentration of approximately 1mg/mL or less. Gently vortex to mix.

Peptide Storage after Reconstitution:

Reconstituted peptide should be aliquoted into several freezer vials and stored at –20°C or lower. Do not freeze thaw. Note: Beta-Amyloid Peptides are shipped as a lyophilized powder at ambient temperature. Upon receipt, store lyophilized powder at –20°C or lower.

Additional Information

**End users are responsible for adopting the solubilization method according to their specific application.*

Listed below are excerpts from product citations using NH_4OH solvent supplied with beta-amyloid peptide for solubilization.

Citation: Saha A, Mondal G, Biswas A, Chakraborty I, Jana B, Ghosh S. In vitro reconstitution of a cell-like environment using liposomes for amyloid beta peptide aggregation and its propagation. Chem Commun (Camb). 2013; 49(55):6119-21.
Excerpt: Aβ peptide aggregation study inside of the liposome: In an eppendrof tube 0.01 mg of Aβ42 peptide and 0.001 mg of Aβ42 with HiLyte Fluor™ 488 labelled peptides were taken as 1,1,1,3,3,3-Hexafluoro-2-propanol solution. The solution was dried by nitrogen flush. A mixture of 200 µL of IB and 0.7 µL of 1% NH_4OH (supplied along with the peptides from Anaspec as a peptide reconstitution solvent) solution was prepared and adjusting its osmolarity with sucrose (2M) solution. 28 µL of this solution was added to the tube containing the Aβ42 peptide mixture.
https://pdfs.semanticscholar.org/8bfd/39eb2db09e001e214145fecf684615987e5a.pdf
http://pubs.rsc.org/en/Content/ArticleLanding/2013/CC/c3cc41287c#!divAbstract

Citation: Lana, Erica, Mahbod Khanbolouki, Charline Degavre, Eva-Britt Samuelsson, Elisabet Åkesson, Bengt Winblad, Evren Alici, Christina Unger Lithner, and Homira Behbahani. "Perforin Promotes Amyloid Beta Internalisation in Neurons." Molecular neurobiology (2016): 1-14.
Excerpt: Aβ Treatment: To investigate the mechanism of Aβ internalisation, we used RA-differentiated SH-SY5Y (RA-SH-SY5Y) cells and hPCN, which were treated with Alexa Fluor 488-labelled Aβ40 or Aβ42 peptides (AnaSpec, Abs/Em: 503/528 nm). The SH-SY5Y cells were differentiated on glass coverslips in 24-well plates as described above. The peptides were prepared according to the manufacturer's instructions: briefly, the peptides were reconstituted by adding 40 µl of 1 %NH_4OH to 0.1 mg of Aβ40 or 50 µl of 1 % NH_4OH to 0.1 mg of Aβ42 peptides labelled with HiLyte Fluor 488. The peptide solution was diluted to approximately 1 mg/ml with PBS, and aliquots were kept at −20 °C. *http://link.springer.com/article/10.1007/s12035-016-9685-9*

Listed below are excerpts from product citations that may provide alternate solubilization methods.

Amyloid β(1–42) peptide was purchased from AnaSpec (San Jose, CA, USA). A 1 mg sample of peptide was dissolved in 200 µL hexafluoroisopropanol (HFIP) and aliquoted to obtain 0.1 mg stocks (handle HFIP in a chemical fume hood taking the necessary precautions) - Reinke, AA. et al. *Chem Biol Drug Design* **70**, 206 (2007).

Synthetic Aβ (1–42) (AnaSpec, San Jose, CA) was prepared for aggregation by resuspending lyophilized Aβ (1–42) in hexafluoroisopropanol, dried under a nitrogen stream, and stored as a film at –20 °C. Immediately prior to use, Aβ (1–42) was resuspended in Me SO to 10mM and sonicated for 10 min. For experiments in which early stages of aggregation were studied, these aliquots were rapidly brought to 25 µM in phosphate-buffered saline (PBS), pH 7.2, and

used immediately. Oligomers were prepared by diluting the Aβ (1–42) to 25 μM with phenol red-free DMEM-F12 and incubating for 24 h at 4 °C without shaking. Fibrils were similarly prepared by incubating 25 μM Aβ (1–42) in PBS at 37 °C for 24 h with vigorous shaking - Evans, CG. et al. *J Biol Chem* **281**, 33182 (2006).

The ability to produce the presumably neurotoxic, aggregated β-sheet structure is dependent on many factors, particularly the peptide concentration ionic strength, solvent polarity. For example, the aggregation rate is extremely rapid in aqueous acetonitrile solutions, such as those used for HPLC purification of the peptide. The longer that the Aβ (1-42) peptide remains in aqueous acetonitrile solution, the more likely it will become an aggregated β-sheet structure. Additionally, different commercially prepared batches of HPLC-purified Ab (1-42) peptides can have different starting aggregations states and structures, which will then in turn affect their solubility, aggregation rates, biological activities in solution and the ability to reproduce biophysical measurements. To partly overcome the above complications, we develop a pretreatment method that involves sonicating the dry peptide in conc. TFA before biophysical measurements. TFA breaks up the pre-aggregated peptides and affords monomeric random coil structures. This method ensures that different batches of purified Ab (1-42) peptide will provide reproducible starting points for biophysical and neurotoxicity studies. - Salomon, AR. et al. *Biochem* **35**, 13568 (1996).

Solubility of Aβ (1-42) is pH and concentration dependent, It is significantly insoluble at pH 7.4; it is highly insoluble in aqueous media but are soluble at 40 mg/ml in the a-helix- promoting solvent, 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, handle HFIP in a fumehood and take the necessary precautions) - Burdick, D. et al. *J Biol Chem* **267**, 546 (1992).

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