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ab120301 Amyloid beta peptide (1-42) (human) protocol

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1. Overview

Amyloid beta is a highly aggregation prone peptide and as a result, it is difficult to obtain peptide that is free of pre-aggregates without a preparation step prior to use. Abcam's amyloid beta 1-42 (ab120301) is supplied lyophilized, and there are several methods in the literature to obtain pre-aggregate free amyloid beta. The most common methods are outlined below. We suggest selecting a method that most closely suits your experimental needs for amyloid beta 1-42 preparation.

The solubility of amyloid beta can also be batch dependent. Please refer to the batch-specific CoA for more information about the solubility of a batch.

Solubility methods

pH/solvent methods

The original standard methods in the literature for the preparation of amyloid beta involve dissolving the amyloid beta in a solution with either high or low pH.

(a) NH_4OH

- Add a small amount of 1% NH_4OH directly to the lyophilized solid (50-100 µl should be sufficient for 1mg of peptide)

- Dilute to a concentration of 1mg/ml or less with your buffer.

- Vortex gently to mix (less than 1 minute).

The peptide cannot be stored long term in 1% NH_4OH, therefore it is important to immediately dilute the NH_4OH/peptide solution with PBS or other buffer to a concentration of 1 mg/ml.

Note: This method may not completely remove pre-aggregates. Vortexing may encourage seeding and further aggregation of the peptide.

(b) Sodium hydroxide

- Dissolve the peptide in 10 mM sodium hydroxide.

- Vortex gently to mix (less than 1 minute).

Note: Sodium hydroxide may not completely remove pre-aggregates. Vortexing may encourage seeding and further aggregation of the peptide.

(c) Organic solvents

These have been used when high or low pH may not be appropriate. Amyloid beta is soluble in DMSO, but reconstitution in DMSO takes time compared to NH_4OH which dissolves the peptide quickly. This method may not completely remove pre-aggregates. However, these methods may be appropriate for experiments where the presence of some pre-aggregates is not particularly problematic.

(d) HFIP

There are protocols that involve the use of HFIP (1,1,1,3,3,3-hexafluoro-2-propanol) to remove pre-aggregates from amyloid beta. Recently, the use of HFIP has been suggested by a number of groups to cause enhanced aggregation. Ryan et al., made a detailed comparison of NH_4OH and HFIP treatment and reported that treatment with NH_4OH produced a much more homogenous and monomeric solution than HFIP (Ryan et al., 2013).

2. Size exclusion chromatography methods

Recent literature (Hellstrand et al., 2010; Johnson et al., 2011) has shown that size exclusion chromatography (SEC) may be a suitable method for the complete removal of aggregates from amyloid beta. Hellstrand et al. purified the peptide using SEC twice but Johnson et al. showed that once is sufficient. Disadvantages to this method include the requirement for a reasonably large amount of material, lowering the concentration on the peptide on the column and a lengthy and expensive preparation step. However, if it is crucial to the experiment that as many pre-aggregates are removed as possible this method may be superior to a pH or solvent method.

3. References

Hellstrand E, Boland B, Walsh DM, Linse S. (2010) Amyloid β-protein aggregation produces highly reproducible kinetic data and occurs by a two-phase process. ACS Chem Neurosci. 1(1):13-8.

Johnson RD, Schauerte JA, Wisser KC, Gafni A, Steel DG. (2011) Direct observation of single amyloid-β(1- 40) oligomers on live cells: binding and growth at physiological concentrations. PLoS One 6(8):e23970.

Ryan TM, Caine J, Mertens HD, Kirby N, Nigro J, Breheney K, Waddington LJ, Streltsov VA, Curtain C, Masters CL, Roberts BR. (2013) Ammonium hydroxide treatment of Aβ produces an aggregate free solution suitable for biophysical and cell culture characterization. PeerJ. 1:e73.

Technical Support

Austria
wissenschaftlicherdienst@abcam.com | 019-288-259
France
supportscientifique@abcam.com | 01.46.94.62.96
Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154
Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com
Deutsch: 043-501-64-24 | Français: 061-500-05-30
UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
ca.technical@abcam.com | 877-749-8807
US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823
China
cn.technical@abcam.com | 400 921 0189 / +86 21 2070 0500
Japan
technical@abcam.co.jp | +81-(0)3-6231-0940
Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450
New Zealand
nz.technical@abcam.com | +64-(0)9-909-7829