SensoLyte® Fluorescent β-Amyloid$_{1-42}$ Sampler Kit

Revision number: 1.1 *Last updated: 11/28/2012*

Catalog #	**72071**
Kit Size	6 vials β-amyloid$_{1-42}$ peptides

This kit provides a total of 6 β-amyloid$_{1-42}$ peptides, including:

- Two unlabeled control peptides
- A biotin labeled β-amyloid$_{1-42}$ peptide
- Three β-amyloid$_{1-42}$ peptides with different fluorophore conjugates

Kit Components, Storage and Handling

Component A: β-amyloid$_{1-42}$ peptides (each as lyophilized powder)

Catalog#	Fluorescent Peptide	Ex/Em (nm)
24224	Unlabeled β-amyloid$_{1-42}$	N/A
25382	Scrambled β-amyloid$_{1-42}$ AIAEGDSHVLKEGAYMEIFDVQGHVFGGKIFRVVDLGSHNVA	N/A
23524	Biotin β-amyloid$_{1-42}$	N/A
23526	FAM-β-amyloid$_{1-42}$	494/521
60479	HiLyte Fluor™ 488-β-amyloid$_{1-42}$	503/528
60476	TAMRA-β-amyloid$_{1-42}$	544/572

Component B: Solvent for β-amyloid$_{1-42}$ (1 mL)

Storage and Handling

- Store all peptides (Component A) at -20°C.
- Keep peptides (Component A) away from light and protect from moisture.
- Component B may be kept at 4°C.

Introduction

Among the pathological hallmarks in the brain with Alzheimer's disease are abundant extracellular deposits of insoluble amyloid β-peptides, Aβ$_{1-40}$ and Aβ$_{1-42}$. These peptides are the products of endoproteolytic cleavage of a large transmembrane amyloid precursor protein (APP).[1,2] The APP is first cleaved by β-secretase near the extracellular face of the membrane, which generates the large, soluble ectodomain (sAPP-β) into the medium and retention of a 99-residue C-terminal fragment (C99) in the membrane. The latter can be further cleaved by γ-secretase in the domain within the lipid bilayer. The γ-secretase can cleave C99 at two sites, more frequently at 12 amino acids and less

frequently at 14 amino acids C-terminal to the extracellular face of the membrane. This results the 40 amino acid Aβ peptide ($A\beta_{1-40}$) and its 42 amino acid counterpart ($A\beta_{1-42}$). Aβ peptides are amphipathic, consisting of 28 hydrophilic amino acids and 12-14 hydrophobic amino acids. Mutations occurring at the β-amyloid N-terminal, such as the Swedish double mutation, appear to increase levels of both $A\beta_{1-40}$ and $A\beta_{1-42}$ by facilitation β-secretase cleavage.

Fluorophore labeled β-amyloid peptides have been used in investigating Aβ's aggregation,[3] microglial activation, phagocytosis,[4] and Aβ generation and clearance. The DHL™ fluorescent β-amyloid$_{1-42}$ sampler kit provides three different fluorophore-labeled $A\beta_{1-42}$ peptides, a biotin labeled $A\beta_{1-42}$ peptide, and two control peptides with normal or scrambled sequences. The peptide sequence of β-amyloid$_{1-42}$ is DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA. For the fluorescently labeled peptides, the fluorophore is attached to the N-terminus.

Sample Protocol

Prepare stock solution

Allow all kit components to warm to room temperature. Add 5 µL of solvent (component B) to each vial of β-amyloid$_{1-42}$, and then add 45 µL deionized water. Dissolve the contents completely. The concentration of the stock solution will be 100 µM. This reconstituted stock solution may be stored at -20°C for up to one week.

Immunofluorescence phagocytosis assay[4]

Dilute the fluorophore labeled β-amyloid stock solution in serum-free culture medium to 10-1,000 nM, and allow it to aggregate for 1 hr at room temperature. The aggregated Aβ is vortexed before being added to microglial culture. The uptake of β-amyloid in the cells can be examined by fluorescent microscope over time, or the cells can be detached and analyzed by flow cytometry.

For other assays, researchers need to establish their own protocols.

References

1. Selkoe DJ (1999). *Nature*, 399, A23-A31.
2. Lam FC (2001). *J. Neurochem*, 76**,** 1121-1128.
3. Frost D, Gorman PM, Yip CM, Chakrabartty A (2003). *Eur.J.Biochem.*. 270, 654-663.
4. Li R (2000). *J. Neurochem*, 75, 1447-1454.