Stable Isotope Labeling Kinetics (SILK™) to Measure the Metabolism of Brain-Derived Proteins Implicated in Neurodegeneration

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Alzheimer's disease (AD) is a progressive neurodegenerative disease on track to becoming one of the greatest challenges to the healthcare system in the 21st century. AD affects millions of people in one way or another. It causes long-term memory loss, confusion, mood swings, and, eventually, loss of bodily functions. Sufferers from Alzheimer's tend to withdraw from family, friends and other members of society as symptoms worsen. To date, there are no known cures, and patients and families of patients struggle with symptoms until death. Recent research has shown hope for early diagnosis and treatment. Much of this research has focused on amyloid plaques that are present in the brains of Alzheimer's patients. One approach to studying this unnatural accumulation of amyloid plaques is to monitor synthesis and clearance of the beta-amyloid peptide (Aβ) using L-Leucine ($^{13}C_6$, 99%) (CLM-2262-H).

Quantifying alterations in protein synthesis and clearance rates is vital to understanding disease pathogenesis. It also enables a determination of the effects of novel drug treatments on target protein metabolism. The powerful combination of *in vivo* stable isotope labeling and mass spectrometry has made this possible. Specifically, researchers at Washington University have developed a proprietary method to measure the metabolism of Aβ and other proteins in the human central nervous system (CNS). C$_2$N Diagnostics, LLC, has commercialized this platform for use in CNS drug development, disease detection and progression monitoring.

In this method, individuals receive an administration of L-Leucine ($^{13}C_6$, 99%) followed by serial cerebrospinal fluid (CSF) and plasma sampling. The clinical site that obtains these biological samples then sends them off to a central laboratory (*i.e.,* at C$_2$N Diagnostics) for processing and analysis. Mass spectrometry quantifies the [$^{13}C_6$] leucine enrichment of Aβ to obtain rates of amyloid production and degradation. The SILK™ platform can also assess the kinetics of apolipoproteinE (apoE) in cell culture as well as the human brain, among other proteins implicated in neuro-degeneration. ApoE is the greatest known genetic risk factor for late-onset Alzheimer's disease. Elucidating the metabolism of the various apoE isoforms is beginning to provide important insights about the role that apoE plays in the disease progression of AD.

The SILK™ platform enables the testing of Alzheimer's drugs *in vivo* to determine the effects of the drug on the CNS and other systems in the body. This information is beneficial as a therapeutic biomarker for use in early clinical development. It has the potential to halt undeserving drug candidates early during the development process; thereby, reducing high downstream costs and wasted time to pharmaceutical companies.

Since most leucine-containing proteins are labeled after [$^{13}C_6$] leucine infusion, this robust and versatile technique can be used as a method to determine the turnover rates for many different proteins. It can identify and quantify potential biomarkers for diseases and metabolic disorders beyond Alzheimer's. Please see the list that follows for peer-reviewed references that describe the utility of this method.
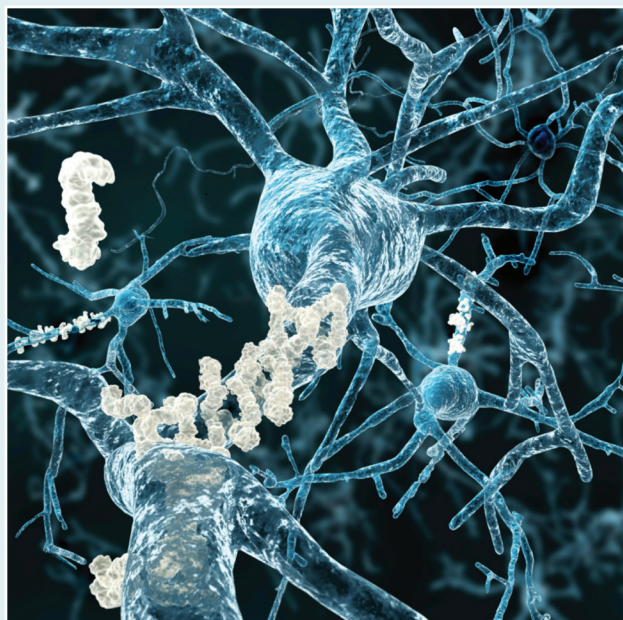
Alzheimer's neurons with amyloid plaque.

SILK™ is a trademark of C$_2$N Diagnostics.

Selected Publications

Huang, Y.; Potter, R.; Sigurdson, W.; Kasten, T.; Connors, R.; Morris, J.C.; Benzinger, T.; Mintun, M.; Ashwood, T.; Ferm, M.; Budd, S.L.; Bateman, R.J. **2012**. β-Amyloid Dynamics in Human Plasma. *Arch Neurol, 69,* 1591-7.

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Wildsmith, K.R.; Han, B.; Bateman, R.J. **2009**. Method for the simultaneous quantitation of apolipoprotein E isoforms using tandem mass spectrometry. *Anal Biochem, 395,* 116-8.

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Bateman, R.J.; Munsell, L.Y.; Chen, X.; Holtzman, D.M.; Yarasheski, K.E. **2007**. Stable Isotope Labeling Tandem Mass Spectrometry (SILT) to Quantify Protein Production and Clearance Rates. *J Am Soc Mass Spectrom, 18,* 997-1006.

Bateman, R.J.; Wen, G.; Morris, J.C.; Holtzman, D.M. **2007**. Fluctuations of CSF amyloid-beta levels: Implications for a diagnostic and therapeutic biomarker. *Neurology, 68,* 666-9.

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Related Product

Catalog No.	Description
CLM-2262-H	L-Leucine ($^{13}C_6$, 99%)