



S-Adenosyl Methionine (SAM) Fluorescence Assay

Easy - Fast - Sensitive - Flexible

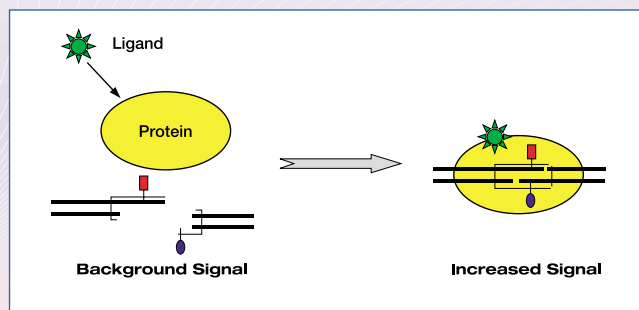
Adaptable to Low and High-Throughput Testing Formats

Measuring DNA-Binding Proteins and Their Ligands

Eukaryotic cells contain an estimated 3,000 sequence-specific DNA-Binding proteins. These important proteins, acting either alone or in combination with a small molecule co-regulator (ligand), control all aspects of genomic DNA activity including gene expression, DNA replication, and DNA repair. Mediomics is applying its novel fluorescence assay platform to develop in vitro assays capable of rapidly and sensitively quantifying DNA-Binding proteins and their small molecule co-regulators (ligands).

Fluorescence Assay Platform Design

The common property of all sequence-specific DNA-Binding proteins is their ability to bind with high affinity and specificity to a DNA duplex containing a unique nucleotide sequence, i.e., the DNA-Binding site for the protein. Mediomics' novel assay platform relies on this common characteristic. A DNA duplex containing the sequence-specific DNA-Binding site for a given target protein is split into two DNA "half-site" duplexes each having a short single-stranded overhang. These single-stranded extensions are short enough so that in the absence of the target protein little spontaneous re-association occurs. When the target protein is present, however, its high affinity for the full-length DNA sequence will drive the re-association of the two half-site DNA duplexes. This re-association can be sensitively detected by incorporating an appropriate fluorescence probe into each one of the two DNA half-sites. The presence of the DNA-Binding protein is detected as a change in fluorescence signal. A simple variation of this basic platform design allows a DNA-Binding protein to function as a sensitive biosensor for its specific small molecule co-regulator (ligand) as represented schematically below:



S-Adenosyl Methionine

S-adenosyl methionine (also referred to as SAM, SAME or AdoMet) plays a crucial role in the biological process of methylation in all types of organisms. In the methylation cycle, SAM serves as the donor of the methyl group used in the covalent modification of DNA and proteins. Variability in SAM levels have been linked to the processes of aging, numerous neurological and psychiatric disorders including Alzheimer's disease, depression, HIV-related neurological dysfunction/dementia, multiple sclerosis, Parkinson's disease, spinal cord degeneration, epilepsy, fibromyalgia, migraine headaches, and also chronic liver dysfunction, arteriosclerosis and cancer. Currently, SAM is quantified using the high pressure liquid chromatography (HPLC) method. HPLC is time consuming, costly and, due to the large amount of organic solvent required, not environmentally friendly.

Bridge-It® S-Adenosyl Methionine Fluorescence (SAM) Assay^{1,2}

Mediomics Bridge-It® S-adenosyl methionine (SAM) fluorescence assay method is based on a combination of well-established fluorescence measurement techniques and our patented new assay platform design that utilizes DNA-binding proteins as biosensors for their respective small molecule co-regulators (ligands). The affinity of



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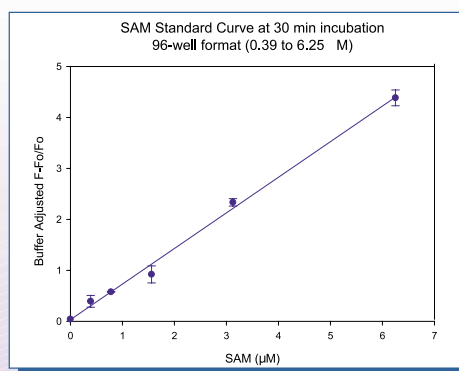
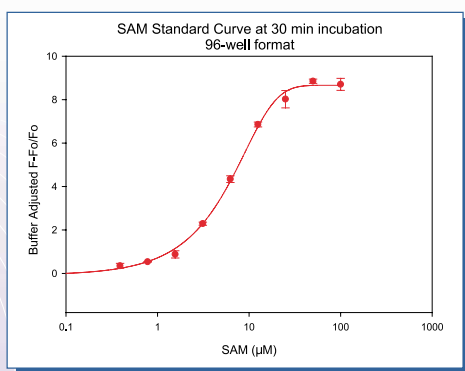


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the DNA sequence-specific MetJ protein for its unique DNA-Binding site is greatly increased in the presence of its ligand, S-adenosyl methionine. For this assay, the MetJ consensus sequence was split into two DNA "half-sites". One half fragment was labeled with fluorescein and the other half fragment was labeled with Oyster® 645 fluorophore. The relative amount of SAM present in a test sample will influence the amount of DNA-MetJ protein complex formation in the assay. When this complex forms, it brings the fluorescence labeled-DNA half-sites into close proximity and causes a measurable change (increase) in fluorescence signal emission that can be readily measured using a microplate reader (wavelength settings: absorption 485 nm; emission 665 nm). SAM concentrations in test samples are then determined using a SAM standard curve. The Bridge-It® SAM fluorescence assay method exhibits highly desirable performance characteristics including a high (>5:1) signal to background (S/B) ratio, a broad linear dynamic range (0.5 μ M - 20 μ M), and, a detection sensitivity of 0.5 μ M. This detection level (0.5 μ M) is useful for quantifying SAM in most test samples of interest including biological fluids, cell culture and fermentation medium, and extracts of tissues and cells.



In contrast to the HPLC procedure, the Bridge-It® SAM fluorescence assay method utilizes the 384 and 96-well microplate format. Thus, it is ideally suited for the rapid, simultaneous measurement of SAM levels in large numbers of test samples. Also, this method is readily adaptable to the high-throughput screening platforms currently being used in drug discovery research. In comparison with the HPLC procedure, the Bridge-It® SAM fluorescence assay method is:

Easy

Mix test sample or standard with the Assay solution and incubate at ~25°C

Fast

Read fluorescent signal after 30 minutes of incubation

Sensitive

Assay measures SAM at a lower limit of sensitivity of 0.5 μ M (i.e., 50 pmol / well in a 96-well or 20 pmol in a 384-well black microplate)

Flexible

Assay is adaptable to both low- and high-throughput screening formats

Additional information on the Bridge-It® S-adenosyl methionine fluorescence assay protocol and product ordering information are available on-line at www.mediomics.com.

1 Bridge- It® is a registered trademark of Mediomics, LLC, St. Louis, Missouri, U.S.A. Mediomics has a worldwide, exclusive license for this assay platform from Saint Louis University, St. Louis, Missouri.

2 The Bridge-It® S-adenosyl methionine (SAM) fluorescence assay kit is provided for laboratory R&D use only. This product is not approved by the U.S. Government or by the government of any other country of the world for use in clinical diagnosis of disease or treatment of disease in humans or animals.

3 Oyster® is a registered trademark of Denovo Biolabels, GmbH, Munster, Germany.

4 Flownamics® Analytical Instruments, Inc., Madison, Wisconsin, is the authorized U.S. distributor of Oyster® dyes.



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