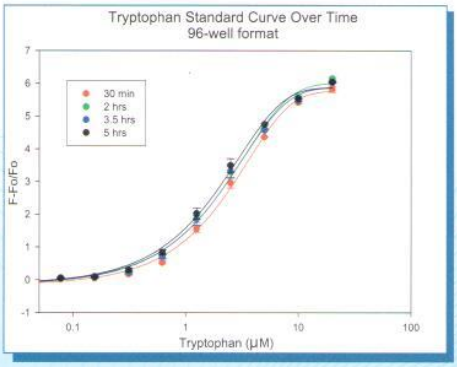
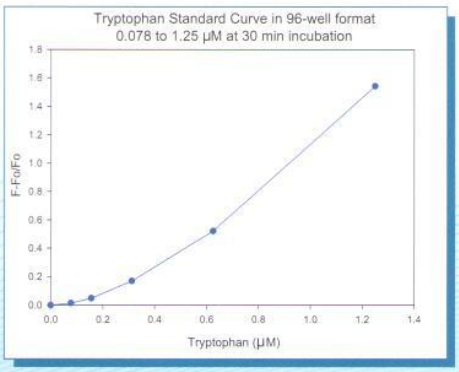
Bridge-It® Tryptophan Fluorescence Assay

Easy - Fast - Sensitive - Flexible

Adaptable to Low and High-Throughput Testing Formats

The central feature of this assay design is the TrpR-dependent association of two fluorochrome-labeled DNA half-fragments (one labeled with fluorescein and the other labeled with Oyster® 645 fluophore[3,4]). Each fragment contains about one-half of the TrpR protein DNA-binding site. In the presence of L-tryptophan an increase in fluorescence signal can be detected as a result of the tryptophan-dependent association of the labeled DNA half-fragments. Tryptophan is readily detectable using the Bridge-It® tryptophan fluorescence assay in various types of test samples including bacterial growth medium, brain extract, yeast extract, as well as in human serum and urine. The linear range of the assay is 0.4 µM-10 µM and the minimum tryptophan detection level is ~ 0.1 µM. The assay is highly specific for measuring tryptophan. No significant TrpR protein binding activity was observed using the assay when L-tryptophan was replaced with each of nineteen (19) other L-amino acids (up to 100 µM) or D-tryptophan, serotonin, and the tryptophan precursor 5'HTP (up to 20 µM).





The Bridge-It® L-tryptophan fluorescence assay is performed using the 96-well or 384-well microplate format and is, therefore, ideally suited for the rapid, simultaneous measurement of tryptophan in large numbers of test samples. In comparison with HPLC, the Bridge-It® tryptophan fluorescence assay is:

Easy

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

Fast

Read fluorescent signal after 30-minute incubation
(reader settings: excitation 485 nm; emission 665 nm)

Sensitive

Assay measures L-tryptophan levels as low as 0.1 µM
(i.e., 10 picomoles /well in a 96-well black microplate or
2 picomoles /well in a 384-well black microplate)

Flexible

Method is adaptable to high-throughput screening formats

A detailed description of the Bridge-It® L-tryptophan fluorescence assay protocol and product ordering information are available on-line at www.mediomics.com.

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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® tryptophan fluorescence assay is intended for laboratory research 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 disease diagnosis or treatment of 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 for Oyster® dyes.

mediomics

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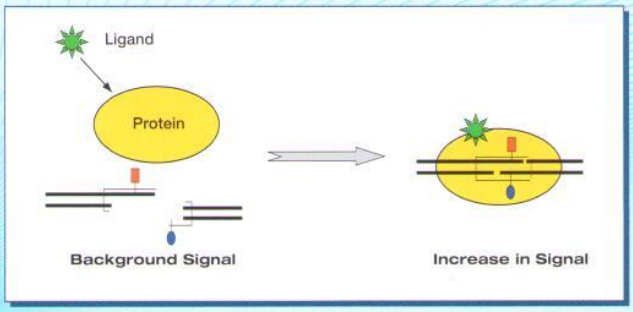
Measuring DNA-Binding Proteins and Their Ligands

Eukaryotic cells contain an estimated 3,000 sequence-specific DNA-binding proteins. These important proteins, acting either with or without a protein-specific small molecule co-regulator (ligand), control all aspects of genomic DNA activity including gene expression, DNA replication, and DNA repair. Mediomics is applying its proprietary fluorescence platform to develop *in vitro* assays capable of rapidly and sensitively quantifying both DNA-binding proteins and their 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 ligand as represented schematically below:



L-Tryptophan

L-tryptophan (tryptophan) is one of eight essential amino acids that must be obtained from the diet. Tryptophan serves as a key building block for synthesis of proteins and as a precursor for various brain neurotransmitters including serotonin. Tryptophan is the only recognized precursor that can be converted into serotonin by the body. Serotonin promotes feelings of well being and calm and thereby helps to counterbalance the physiological affects of brain dopamine and the nor-adrenaline circuits which encourage fear, anger, tension, aggression, obsessive-compulsive actions, over-eating (especially of carbohydrates), migraine headache, depression, and sleep disturbances. Melatonin, a metabolite of serotonin, is a sleep promoting natural hormone made by the pineal gland. In addition, tryptophan is a key precursor for niacin (vitamin B3), a vitamin that is essential for normal respiration, metabolism, and synthesis of sex hormones. Because tryptophan plays such a critical role in the proper balancing of metabolism, mood and sleep patterns, insufficient dietary availability of this essential amino acid can lead to serious adverse consequences.

Bridge-It® L-Tryptophan Fluorescence Assay[1,2]

The Bridge-It® tryptophan fluorescence assay is based on the activity of tryptophan repressor protein (TrpR), a bacterial DNA-binding protein. TrpR protein binds to its DNA-binding site in tryptophan-dependent fashion.

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