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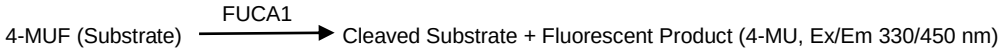
α-L-Fucosidase Activity Assay Kit (Fluorometric)

(Catalog # K542-100; 100 assays; Store at -20°C)

I. Introduction:

α-L-Fucosidase (EC 3.2.1.51) (FUCA1) is a hydrolase that is able to cleave the α-L-fucosyl moieties from glycoconjugates and oligosaccharides. Fucosidase plays a pivotal role in cell differentiation, apoptosis, inflammation and host-pathogen interaction. Furthermore, abnormal concentrations of this glycosidase have been observed in patients suffering from cancer, fucosidosis, rheumatoid arthritis, cystic fibrosis, and leukocyte adhesion deficiency. Industrial applications of FUCA1 include synthesis of fucosylated analogs that could serve as antiadhesion compounds, cancer vaccines, and anti-inflammatory therapeutics. BioVision's α-L-Fucosidase Assay Kit provides a simple, sensitive and high-throughput adaptable approach to detect physiological concentrations of this glycosidase in a variety of biological samples. In this assay, FUCA1 uses a synthetic 4-MUF substrate and releases a fluorescent methylumbelliferyl derivative (4-MU) that can be measured kinetically under acidic conditions (Ex/Em = 330/450 nm). The assay is a single-step reaction, with minimal sample preparation. The assay can detect less than 1 µU/ml of FUCA1 activity in serum samples.



II. Applications:

- Evaluation of FUCA1 activity as a biomarker in serum to detect cancer or other pathology.

III. Sample Types:

- Biological fluids such as serum or plasma.

IV. Kit Contents:

Components	K542-100	Cap Code	Part Number
FUCA1 Assay Buffer	25 ml	WM	K542-100-1
4-MUF Substrate (in DMSO)	55 µl	Blue	K542-100-2
DTT (1 M)	1.0 ml	Green	K542-100-3
FUCA1 Positive Control	20 µl	Orange	K542-100-4
4-MU Standard (5 mM in DMSO)	35 µl	Yellow	K542-100-5

V. User Supplied Reagents and Equipment:

- Multi-well fluorescence microplate reader
- 96-well clear microtiter plates with flat bottom

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VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge vials prior to opening. Read entire protocol before performing the assay.

- **FUCA1 Assay Buffer (AB), DTT (1M):** Store at -20°C or 4°C. Bring to room temperature before use. Aliquot DTT (1M) before storage.
- **4-MUF Substrate (in DMSO) and 4-MU Standard (5 mM in DMSO):** Light sensitive. Aliquot and store at -20°C. Allow reagents to equilibrate to RT before use.
- **FUCA1 Positive Control:** Aliquot and store at 4°C. Protect from light. Do not freeze. Keep on ice during use. Use within 6 months.

VII. Fucosidase Assay Protocol:

1. **Sample Preparation:** Serum and plasma samples can be assayed directly. Add 1-10 µl undiluted sample to a 96-well plate. Add DTT to FUCA1 Assay Buffer at a final concentration of 2 mM. For Positive Control**:** dilute FUCA1 1:1000 by adding 2 µl of FUCA1 to 1998 µl Assay Buffer. Add 10 µl of FUCA1 dilution into the appropriate well(s). Adjust the volume of Positive Control and sample wells to 50 µl/well with FUCA1 Assay Buffer (with DTT).

Notes:

   a. Always prepare fresh FUCA1 Assay Buffer (with DTT) and use within 24 hrs. Keep on ice during use.

   b. For unknown samples, we strongly recommend doing a pilot experiment. Test several doses of sample to ensure values are within the linear range of the standard curve.

   c. For samples having high background, prepare parallel sample well(s) as sample background control. Adjust the volume to 100 µl with FUCA1 Assay Buffer (with DTT).

   d. It is not necessary to dilute serum if sample volume used lies within the dynamic range.

2. **Standard Curve Preparation:** Add 3.0 µl of 5 mM 4-MU Standard to 997 µl of AB to make a 15 µM 4-MU solution for Standard Curve. Add 0, 2, 4, 6, 8, 10 µl of 15 µM 4-MU Standard to generate 0, 30, 60, 90, 120, 150, pmoles of 4-MU/well. Adjust the volume to 100 µl/well with FUCA1 Assay Buffer (with DTT). *Standard Curve can be read in end point mode (i.e. at the final incubation time)*.
3. **Reaction Mix**: Dilute FUCA1 Substrate to working concentration (1:100) by adding for an example 5 µl of stock to 495 µl of FUCA1 Assay Buffer (with DTT). Add 50 µl working FUCA1 Substrate into sample well(s). Prepare sufficient amount for the number of samples that will be tested. Mix well.
4. **Measurement:** Measure fluorescence at Ex/Em 330/450 at 37°C in kinetic mode for 30 minutes. Choose two time points (t_1 and t_2) in the linear range to calculate the slope of each assayed well. Slopes for Standards, background, and samples should be calculated using same time points.

5. **Calculation:** Subtract 0 4-MU Standard reading from all standard readings. Plot the 4-MU Standard Curve; apply Sample ΔRFU and Sample Background Control ΔRFU to 4-MU Standard Curve to obtain the corresponding amount of 4-MU formed. Calculate the background-corrected sample ΔRFU (B, in pmol) by subtracting the amount of 4-MU formed by Sample Background Control from the amount of 4-MU formed by the Sample and calculate the activity of FUCA1 in the sample as:

$$\textbf{Sample FUCA1 Activity} = \frac{\mathbf{B}}{\Delta \mathbf{t} \, \mathbf{X} \, \mathbf{V}} \; \mathbf{X\,D} = \mathbf{pmol/min/ml} = \mu\mathbf{U/ml}$$

Where: **B**: is amount of generated 4-MU by FUCA1 from Standard Curve (pmol)
Δt: reaction time (min).
V: is sample volume added into the reaction well (ml)
D: is sample dilution factor.

Unit Definition: One Unit of FUCA1 activity is the amount of enzyme that generates 1 pmole of 4-MU per min at 37°C.

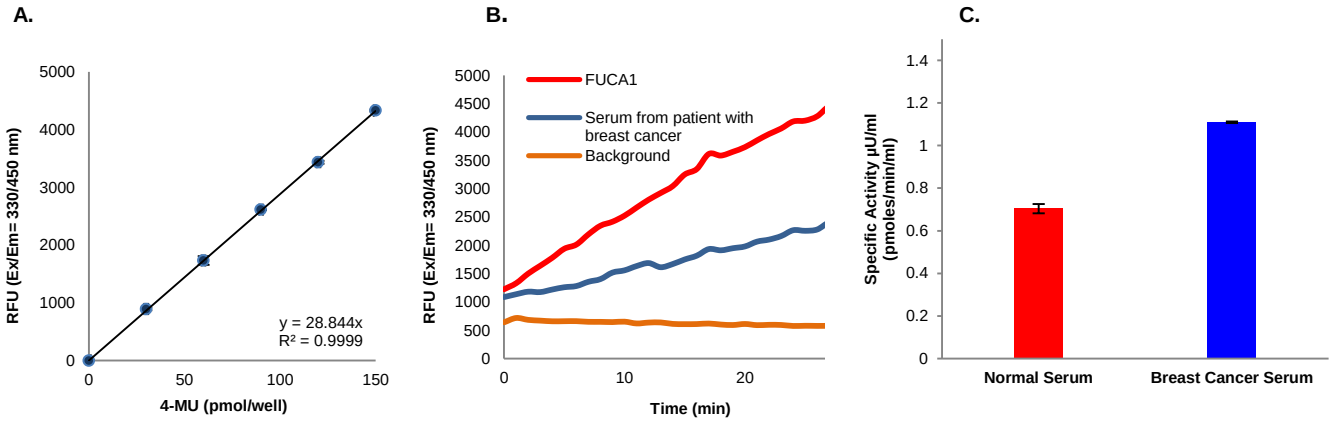

Figure: (**A**) 4-MU Standard Curve (**B**) Measurement of activity in FUCA1, serum from normal patient and serum from breast cancer patient; and background contributed by assay buffer. (**C**) Estimation of FUCA1 in human serum from normal patient and patient with breast cancer. Five microliters of each undiluted sample were assayed following kit protocol.

VIII. RELATED PRODUCTS:

Alanine Aminotransferase (ALT or SGPT) Activity Colorimetric/Fluorometric Assay Kit (K752)
Aspartate Aminotransferase (AST or SGOT) Activity Colorimetric Assay Kit (K753)
Gamma Glutamyl Transferase (GGT) Activity Colorimetric Assay Kit (K784)
Gamma Glutamyl Transferase (GGT) Activity Fluorometric Assay Kit (K785)
Adenosine Deaminase Activity Assay Kit (Colorimetric) (K321)
Adenosine Deaminase Activity Assay Kit (Fluorometric) (K328)
Bilirubin (Total and Direct) Colorimetric Assay Kit (K553)
Sodium deoxycholate (2830)
Chenodeoxycholic acid (2831)
Lithocholic acid (2187)

FOR RESEARCH USE ONLY! Not to be used on humans.