Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

α-L-Fucosidase (FUCA1) Assay Kit (Colorimetric)

(Catalog # K224-100; 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 α-L-fucosyl moieties from glycoconjugates and oligosaccharides. Fucosidase plays pivotal roles 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 α-L-Fucosidase (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, α-L-Fucosidase uses a synthetic chloro p-nitrophenol derivative (R-pNP) as an α-L-Fucosidase substrate and releases a chlorinated pNP derivative which can be measured kinetically under acidic conditions (OD 405 nm). The assay involves a one-step simple reaction, with minimal sample preparation and does not need a stop solution to complete the reaction. The assay can detect as low as 20 µU of α-L-Fucosidase activity in a variety of samples.

$$\text{R-}p\text{NP} + H_2O \xrightarrow{\text{Fucosidase; [H+]}} \text{R} + p\text{NP (OD: 405 nm)}$$

II. Application:

- Estimation of FUCA1/AFU in various biological samples.

III. Sample Types:

- Biological fluids such as serum, plasma, saliva, etc.

IV. Kit Contents:

Components	K224-100	Cap Code	Part Number
FUCA1 Assay Buffer	7.0 ml	NM	K224-100-1
FUCA1 Substrate	6.0 ml	NM/Amber	K224-100-2
pNP Standard (10 mM)	0.75 ml	Red	K224-100-3
DTT (1 M)	0.25 ml	Green	K224-100-4
FUCA1 Positive Control	20 µl	Orange	K224-100-5

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer

VI. Storage Conditions and Reagent Preparation:

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

- **FUCA1 Assay Buffer, FUCA1 Substrate, *p*NP (10 mM), and DTT (1 M):** Store at -20°C or 4°C. Bring to room temperature (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 saliva samples can be assayed directly. Add 5-50 µl undiluted sample to a 96-well plate. For Positive Control, add 1-3 µl of Positive Control into desired well(s). Add DTT to FUCA1 Assay Buffer at a final concentration of 2 mM. Make as much as needed. 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 while in use.

   b. For unknown samples, we strongly recommend diluting the samples, doing a pilot experiment and testing several doses to ensure the readings are within the Standard Curve range.

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

2. **Standard Curve Preparation:** Add 0, 4, 8, 12, 16, and 20 µl of 10 mM pNP Standard into a series of wells in a 96-well plate to generate 0, 40, 80, 120, 160 and 200 nmol of pNP/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:** Add 50 µl FUCA1 Substrate into sample well(s). Mix well.

4. **Measurement:** Incubate plate at 37°C for 60 min., protected from light. Measure absorbance at 405 nm in a kinetic mode. Choose two time points (T_1 & T_2) in the linear range to calculate the slope of every assayed well. Slopes for Standards, backgrounds, and samples should be calculated using same time points.

   Note:

   Incubation time depends on the FUCA1 Activity in the samples.

5. **Calculation:** Subtract 0 pNP Standard reading from all standard readings. Plot the pNP Standard Curve. If sample background control reading is high, subtract it from the sample reading. Calculate FUCA1 Activity of the test sample(s): ΔOD: $OD_1 - OD_2$. Apply the OD to the pNP Standard Curve to get B nmol of pNP generated by FUCA1 Activity during the reaction time (ΔT: $T_1 - T_2$). Calculate FUCA1 activity by following equations:

Sample FUCA1 Activity = B/(ΔT X V) X D nmol/min/ml or mU/ml

Where: **B** is amount of generated pNP by FUCA1 from Standard Curve (nmol)
ΔT: reaction time (min).
V is sample volume added into the reaction well (ml)
D is sample dilution factor

FUCA1 Activity can also be expressed in mU/mg protein.
Unit Definition: One Unit of FUCA1 activity is the amount of enzyme that generates 1.0 nmol of pNP per min. at 37 °C.

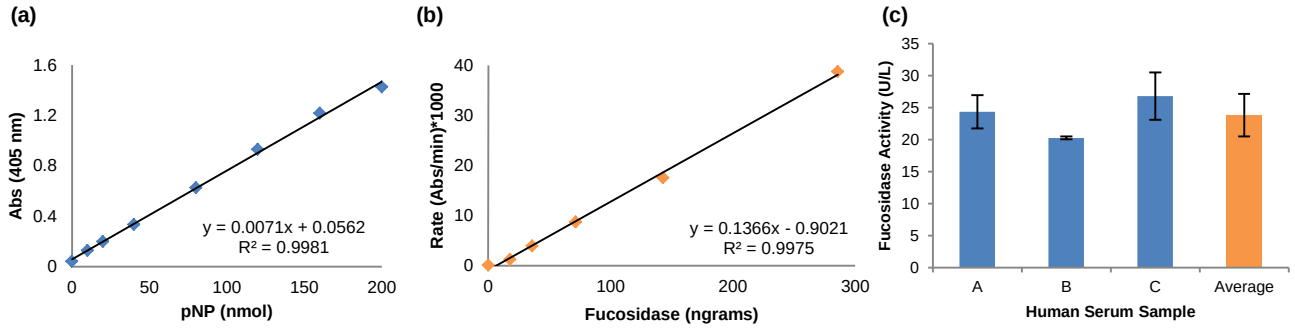

Figure: (a) *p*NP Standard Curve. **(b)** Estimation of FUCA1 rates using the enzyme as a positive control. **(c)** Estimation of FUCA1 in human serum. Fifty microliters of each undiluted sample was assayed following kit protocols. Fucosidase Activity in Pooled Human serum (in U/L): A: 24.3 ± 2.6, B: 20.3 ± 0.25, C: 26.8 ± 3.7, Average of three samples: 23.8 ± 3.3.

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), Fluorometric Assay Kit (K785)
Adenosine Deaminase Activity Assay Kit (Colorimetric) (K321), (Fluorometric) (K328)
Adenosine Deaminase Activity Assay Kit Bilirubin (Total and Direct) Colorimetric Assay Kit (K553)
Sodium deoxycholate (2830)
Chenodeoxycholic acid (2831)
Lithocholic acid (2187)

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