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ab273324 Alpha-L-Fucosidase Activity Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273324
(use https://www.abcam.cn/ab273324 for china, or
https://www.abcam.co.jp/ab273324 for Japan)

For the determination of FUCA1 activity in biological fluids.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Alpha-L-Fucosidase Activity Assay Kit (Fluorometric) (ab273324) 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, Alpha-L-Fucosidase (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.

2. Protocol Summary

Prepare all reagents, samples and positive controls.

Prepare standards.

Add all samples to the appropriate wells.

Add Reaction Mix to the appropriate wells.

Measure fluorescence in kinetic mode for 30 mins at 37°C.

Determine Alpha-L-Fucosidase activity using equation.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
FUCA1 Assay Buffer	25 mL	-20°C
4-MUF Substrate (in DMSO)	55 µL	-20°C
DTT (1 M)	1 mL	-20°C
FUCA1 Positive Control	20 µL	-20°C
4-MU Standard (5 mM in DMSO)	35 µL	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

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

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 **FUCA1 Assay Buffer:**
Store at -20 °C or 4 °C. Bring to room temperature before use.

9.2 **DTT (1M):**
Store at -20 °C or 4 °C. Bring to room temperature before use. Aliquot before storage.

9.3 **4-MUF Substrate (in DMSO):**
Light sensitive. Aliquot and store at -20°C. Allow reagents to equilibrate to RT before use.

9.4 **4-MU Standard (5 mM in DMSO):**
Light sensitive. Aliquot and store at -20°C. Allow reagents to equilibrate to RT before use.

9.5 **FUCA1 Positive Control:**
Aliquot and store at 4°C. Protect from light. Do not freeze. Keep on ice during use. Use within 6 months.

10. Sample Preparation

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

Tissue/cell lysate preparation:

10.1 Serum and plasma samples can be assayed directly. Add 1 - 10 µl undiluted sample to a 96-well plate.

10.2 Add DTT to FUCA1 Assay Buffer at a final concentration of 2 mM.

10.3 **For Positive Control**: dilute FUCA1 1:1000 by adding 2 µl of FUCA1 to 1998 µl Assay Buffer.

10.4 Add 10 µl of FUCA1 dilution into the appropriate well(s).

10.5 Adjust the volume of Positive Control and sample wells to 50 µl/well with FUCA1 Assay Buffer (with DTT).

Δ Note: 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.

Δ Note: 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).

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

11. Standard Curve

11.1 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.

11.2 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.

11.3 Adjust the volume to 100 µl/well with FUCA1 Assay Buffer (with DTT).

Standard #	15 µM 4-MU - Standard (µL)	4-MU Assay Buffer (µL)	4-MU (pmol/well)
1	10	90	150
2	8	92	120
3	6	94	90
4	4	96	60
5	2	98	30
6	0	100	0

Δ Note: Standard Curve can be read in end point mode (i.e. at the final incubation time).

12. Assay Procedure

Reaction mix:

12.1.1 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).

12.1.2 Add 50 µl working FUCA1 Substrate into sample wells. Prepare sufficient amount for the number of samples that will be tested. Mix well.

12.1.3 Measure fluorescence (Ex/Em = 330/450 nm) in kinetic mode for 30 mins at 37°C. Choose two time points (t1 and t2) 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.

13. Calculations

13.1 Subtract 0 4-MU Standard reading from all standard readings.

13.2 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.

13.3 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:

$$Sample\ FUCA1\ activity\ = \frac{B}{(\Delta t\ x\ V)}\ \times D = \ pmol/min/mL = \ \mu U/mL$$

B = 4-MU by FUCA1 from Standard Curve (pmol)
Δt = reaction time (min).
V = sample volume added into the reaction well (ml)
D = 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.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

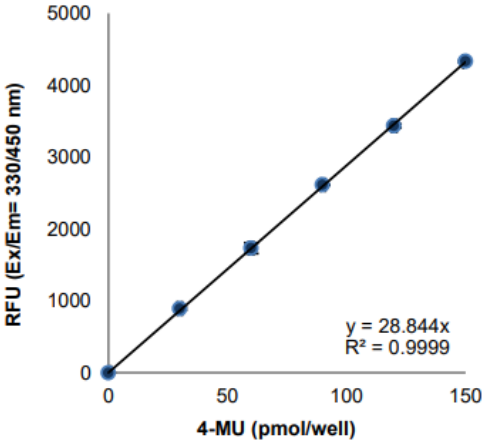

Figure 1. 4-MU Standard Curve.

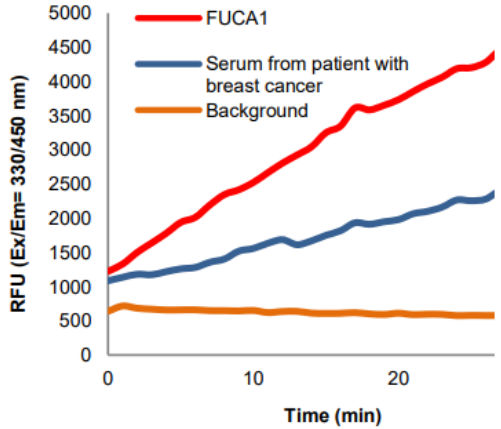

Figure 2. Measurement of activity in FUCA1, serum from normal patient and serum from breast cancer patient; and background contributed by assay buffer.

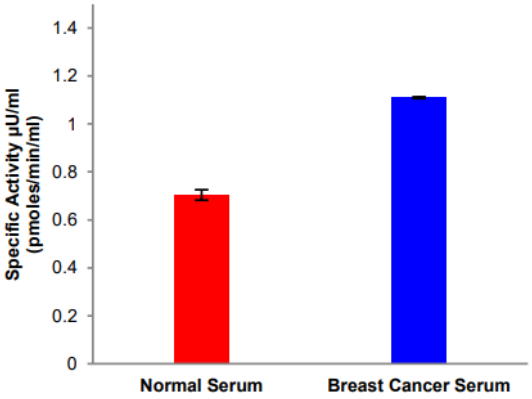

Figure 3. Estimation of FUCA1 in human serum from normal patient and patient with breast cancer. Five microliters of each undiluted sample were assayed following kit protocol.

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)