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α-L-Fucosidase (AFU) Assay Kit
 Catalog Number: BQ 082A-EALD

Intended Use

The α-L-fucosidase (AFU) assay kit is for the determination of AFU activity in patient serum samples. For investigational use or export only.

Clinical Significance¹⁻³

AFU is a lysosomal enzyme involved in the degradation of a diverse group of naturally occurring fucoglycoconjugates. Serum AFU activity is considered a useful marker of hepatocellular carcinoma (HCC). Increased AFU levels in serum are an early indication of HCC. Though measurement of serum fetoprotein (AFP) is a common practice for early detection of HCC, AFP assay alone suffers from its low specificity and sensitivity due to the fact that not all HCC secrete AFP. AFP levels may be normal in as many as 40% of patients with early HCC and 15-20% patients with advanced HCC. Recent studies clearly demonstrated that measurements of both AFP and AFU could significantly increase the detection specificity and sensitivity for HCC. AFU is reported to be a more sensitive marker especially for detecting a small tumor size of HCC.

Assay Principle

The AFU assay is based on the enzymatic cleavage of the synthetic substrate 2-chloro-4-nitrophenyl-α-L-fucopyranoside to α-L-fucoside and 2-chloro-4-nitrophenol, which is quantified by measuring the absorbances at 405 nm in a kinetic fashion. It is a one step assay with a single assay reagent. One unit of AFU is defined as the amount of AFU that cleaves one μmole of 2-chloro-4-nitrophenyl-α-L-fucoside per min at 37 °C.

Reagent Composition

Reagent 1 (R1) 2 x 25 mL	100 mM phosphate CNP-AFU substrate
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Reagent Preparation

Reagents are supplied ready-to-use.

Reagent Stability and Storage

Reagent is stable until the expiration date on the label when stored at 2 – 8 °C shielded from light.

Materials Required But Not Provided

An analyzer capable of dispensing a minimum of 1 reagent and of measuring absorbance at 405 nm with temperature control (37°C).

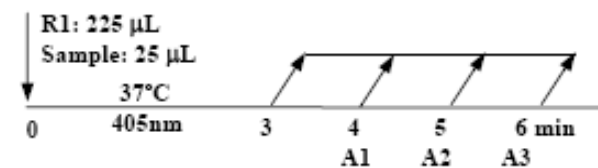
Specimen Collection and Handling

Use fresh and non-hemolyzed serum for AFU assay. AFU is stable in serum for one week at 4°C.

Precautions

1. Specimens and reagents containing human sourced materials should be handled as if potentially infectious, using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395).
2. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
3. Avoid swallowing and contact with skin or mucous membranes.

Assay Procedure



Method: Kinetics
 Wavelength: 405nm
 Reference wavelength: 505 nm
 Reagent (R1) blank (autozero) at 405 nm
 Temperature: 37 °C
 Reaction Time: 8 min
 Sample/Reagent: 1: 9

Assay: (one step assay)

- Bring Reagent R1 to room temperature prior to running the assay.
- Mix 225 μL of R1 and 25 μL of plasma sample.
- Incubate at 37 °C for 3 min, followed by measuring the absorbance increase at 405 nm for 1, 2, and 3 min.
- Calculate the average rate of the absorbance change

$$\Delta A/\text{min} = \frac{\Delta A_1/\text{min} + \Delta A_2/\text{min} + \Delta A_3/\text{min}}{3}$$

Note:

Before performing the assay in lab instrument or analyzer, users should verify the accuracy of the calculation factor. The calculation Factor for UV spectrophotometer is **1250** when the cuvette path length is 1 cm. Users should determine the calculation factor for the specific instrument being used in the lab based on cuvette pathlength and other conditions. This can be done experimentally as follows:

- 1) Bio-Quant controls with known values are run in triplicate
- 2) The calculation factor is modified so that the result matches Bio-Quant control target values

Calibration

A single calibrator (to be purchased separately) is needed for running the assay in calibration mode.

Quality Control

We commend that each laboratory use AFU controls to validate the performance of AFU reagents. The range of acceptable control limits should be established by individual laboratories.

Results

Assay results can be obtained in two alternate ways.

- 1) By use of a calibrator
- 2) Alternatively by use of a factor for calculating activity.

Assay Results by Factor Method

- Calculate the average rate of the absorbance change ΔA/min.

$$\Delta A/\text{min} = \frac{\Delta A_1/\text{min} + \Delta A_2/\text{min} + \Delta A_3/\text{min}}{3}$$

- Calculate AFU activity (U/L) in the plasma sample by using the formula:

$$\text{AFU (U/L)} = \frac{\Delta A/\text{min} \times Tv}{\epsilon \times Sv \times L} = \Delta A/\text{min} \times 1250$$

ε: μmolar extinction coefficient of dye
 Tv: Total reaction volume (mL)
 Sv: Sample volume (mL)
 L: Cuvette light path length (1.0cm)

Limitations

If the sample AFU activity is greater than 300 U/L, the sample should be diluted with saline before measurement. The result should be multiplied by the dilution factor.

Reference Range

Healthy subjects have an AFU activity in the range of 0–40 U/L, or 0-667 nkat/L. Attention should be paid to samples from pregnant women whose serum AFU activity may be elevated. It is recommended that each laboratory should establish its own range of reference values.

Performance Characteristics**Linearity**

The assay is linear from 0-300 U/L (37 °C). $r^2 > 0.99$

Precision: Intra assay CV% < 5.1%, Inter assay CV% < 6.2%

Interference

Assay is not affected by serum bilirubin up to 100mg/dL, hemoglobin up to 200 mg/dL, triglycerides up to 750mg/dL, and ascorbic acid up to 4.4mg/dL.

References

1. Zielke K. et al. Fucosidosis: diagnosis by serum assay of α -L-fucosidase. J. Lab. Clin. Med. 79: 164 (1972)
2. Giardina MG. et al. Serum α -L-fucosidase. A useful marker in the diagnosis of hepatocellular carcinoma. Cancer, 70: 1044 (1992)
3. Ayde D. et al. Value of the serum α -L-fucosidase activity in the diagnosis of colorectal cancer. Oncology, 59: 310 (2000)

Cobas Mira Parameters		AFU Kinetics
Measurement Mode		Absorb
Reaction Mode		R-S
Calibration Mode		Factor
Reagent Blank		Reag/DIL
Cleaner		No
Wavelength		405 nm
Decimal position		2
Unit		U/L
Sample cycle		1
Sample volume		25.0 μ L
Sample dilution		H ₂ O
Dilution volume		0.0 μ L
Reagent cycle		1
Reagent volume		225 μ L
Sample limit		No
Reaction direction		Increase
Convers. factor		1.0000
Offset		0.0000
Test range Low		0.000 U/L
Test range High		300.00 U/L
Number of steps		1
Calc. Step A		Kinetics
Readings first		10
Readings last		18
Calibration		
Calibration interval		Each day
Time		No
Blank		
Reagent range	low	-0.1
	high	0.6
Blank range	low	-0.1
	high	0.1
Factor		2500

Attention: * Each reading cycle is 25 seconds.

Hitachi 717 Parameters in Factor Mode

Test	AFU
Assay Code	Rate-A
Assay Point	(25)-(40)**
Wavelength	750/405
Calibration Method	K Factor
Unit	U/L
Sample volume	(20)(20)
Reagent vol. R1	(225)(100)(NO)
Reagent vol. R2	(0)(0)(NO)
STD. (1) CONC.-Position	(0)-(1)* Blank
STD. (2) CONC.-Position	(0)-(0)
K Factor	2150
ABS Limit	32000-Increase
SD Limit	(0.1)
Duplicate Limit	100
Sensitivity Limit	(0)
Expected value (normal Value)	0-999 U/L
Instrument Factor	1
Tech. Limit	0-999

Hitachi 717 Parameters in Calibration Mode

Test	AFU
Assay Code	Rate-A
Assay Point	(25)-(40)**
Wavelength	750/405
Calibration Method	LINEAR
Unit	U/L
Sample volume	(20)(20)
Reagent vol. R1	(225)(100)(NO)
Reagent vol. R2	(0)(0)(NO)
STD. (1) CONC.-Position	(0)-(1)
STD. (2) CONC.-Position	(*)-(2)
ABS Limit	32000-Increase
SD Limit	(0.1)
Duplicate Limit	100
Sensitivity Limit	(0)
Expected value (normal Value)	0-999 U/L
Instrument Factor	1
Tech. Limit	0-999

**Olympus AU400 Parameters in Calibration Mode
(Temp = 37°C)**

General				
Test Name: AFU	Type: Serum	Operation: Yes		
Sample Volume 20.0 µL	Dilution 0 µL	Pr-Dilution Rate 1		
Reagents:		Min OD	Max OD	
R1 volume 225 µL	Dilution 0 µL	L: -2.000	H: 2.500	
R2 volume 0 µL	Dilution 0 µL			
Wavelength: Pri. 410	Sec. 7 00	Reagent OD Limit:		
Method: Rate		First L: -2.000; First H: 2.500		
Reaction Slope: +		Last L: -2.000; Last H: 2.500		
Measuring Point 1: First 16; Last 26		Dynamic Range:		
Measuring Point 2: First ; Last		L: 0.0	H: 200.0	
Linearity 20%		Correlation Factor:		
No-Lag-Time: No		A: 1.0000	B: 0.000	
Onboard stability Period: 999				
Calibration Type AB		Formula: Y=AX+B		
Counts 2	Process CONC			
Cal No.	OD	CONC	Factor/OD-L	Factor/OD-H
Point 1		*		
Point 2				
MB	Type Factor:		Advanced Calibration: No	
			Calibration Stability Period: 999	

*Entered by operator

**Olympus AU400 Parameters in Factor Mode
(Temp = 37°C)**

General				
Test Name: AFU	Type: Serum	Operation: Yes		
Sample Volume 20.0 µL	Dilution 0 µL	Pr-Dilution Rate 1		
Reagents:		Min OD	Max OD	
R1 volume 225 µL	Dilution 0 µL	L: -2.000	H: 2.500	
R2 volume 0 µL	Dilution 0 µL			
Wavelength: Pri. 410	Sec. 7 00	Reagent OD Limit:		
Method: Rate		First L: -2.000; First H: 2.500		
Reaction Slope: +		Last L: -2.000; Last H: 2.500		
Measuring Point 1: First 20; Last 27		Dynamic Range:		
Measuring Point 2: First 0; Last 0		L: 0.0	H: 200.0	
Linearity 20%		Correlation Factor:		
No-Lag-Time: No		A: 1.0000	B: 0.000	
Onboard stability Period: 999				
Calibration Type MB		Formula: Y=AX+B		
Counts 2	Process CONC			
Cal No.	OD	CONC	Factor/OD-L	Factor/OD-H
Point 1				
Point 2				
MB	Type Factor: 2100		Advanced Calibration: No	
			Calibration Stability Period: 999	