α-L-Fucosidase Assay Kit (AFU)

Method: Colorimetric (Kinetic)

Cat . No.	Size	Instrument
GB9070G	R1:1x60ml R2:1x20ml	For Hitachi 7060/7150 &ShimadzuCL7200/8000
GB9070G/B	R1:2x60ml R2:2x20ml	For Hitachi 7060/7150 &ShimadzuCL7200/8000
GS9071G GK9071G	R1:1x60ml R2:2x20m	For Hitachi7170/7180 &OlympusAU640/400/600
GS9071G/B	R1:2x60ml R2:2x20m	For Hitachi7170/7180 &OlympusAU640/400/600
GX9071G	R1:1x60ml R2:1x20m	For Beckman
GT9071G	R1:1x45ml R2:1x15m	For TOSHIBA 40
GH9071G	R1:1x45ml R2:1x15m	For Hitachi7020
GD9071G	R1:24x3.8ml R2:12x2.6ml	For DATE DIMENSION

INTENDED USE

For the *in vitro* quantitative determination of AFU in serum or plasma .

CLINICAL SIGNIFICANCE

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 can 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.

PRINCIPLE

The AFU assay is based on the enzymatic cleavage of the synthetic substrate 2-chloro-4-nitrophenyl-α-L-fucopyranoside toα-L-fucoside and 2-chloro-4-nitrophenol which is quantified by measuring the absorbances at 405nm 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℃.

SAMPLE COLLECTION AND PREPARATION

Use fresh and non-hemolyzed serum or plasma for AFU test. AFU is stable for one week at 4-8℃.

REAGENT COMPOSITION

Contents	Concentrations in the Test
R1: Good's diluent; stabilizer	100.0mmol/L
R2: phosphate containing substrate (CNP-AFU)	40.0mmol/L

STABILITY AND PREPARATION OF REAGENTS

All reagents are ready to use.
Stable up to the expiry date when stored at 2-8°C.
The AFU assay kit reagents are stable for 1 month after opening and kept at 2-8℃.

ASSAY PROCEDURE

Assay Mode: Kinetics
Wave Length (main/sub): 405 nm/ 660 nm

Sample: 10 μl
R1: 180 μl
R2：60 μl

1. Mix 10μl sample with 180 μl R1 and incubate at 37℃ for 5 minutes；
2. Add R2 60 μl in mixture, and wait for 150 seconds in 37℃；
3. Read initial absorbance and start timer simultaneously, read again after 1 and 2 minutes；
4. Calculate absorbance change per minute (ΔA/min).

1）Assay condition

Main wavelength	405nm	Sample	10ul
Sub wavelength	660nm	R1	180ul
temperature	37℃	R2	60ul
Cuvette light path	1cm	Assay Mode	Rate（+）

2）PROCEDURE

	Blank	Sample
Distilled water (ul)	10	_
Sample volume (ul)	_	10
Reagent1 (ul)	180	180
Sample+R1 mix，incubate for 5minutes at 37℃；then add R2		
Reagent 2 (ul)	60	60
S+R1+R2 mix，incubate for 150 seconds at 37℃，then read △A within 2 minutes，calculate △A/min		

Calibration Procedure

A. Using deionized water and calibrator to do the calibration.
B. The instrument calibration program please refer to instrument user manual.
C.The calibration and calibration frequency requirements: under

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normal circumstances, the calibration should be determinated every 28 days.When the following situations occurs (such as: new reagent batch number, Instrument repairs, maintenance or key parts of the instrument are changed, or the control occurs drifting or beyond the prescribed scope, etc.), the calibration should be renewed determination before patients samples testing.

QUALITY CONTROL

For quality control, use AFU Control as daily quality control sera and can be purchased separately. Values should fall within a specific range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check reaction temperature.
3. Check expiration date of kit and contents.
4. Check the quality of the water used for reagents reconstitution.

REFERENCE VALUE

Healthy subjects have a AFU activity in the range of 0–40 U/L（37℃）;

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.

SPECIFIC PERFORMANCE CHARACTERISTICS

1.Appearance

Reagent R1 is clear colorless liquid, should not exist any flocculent precipitation and sludge in visual; Reagent R2 is clear colorless liquid, should not exist any flocculent precipitation and sludge in visual. Kit label shows clearly, the package is intact. Calibrator and control is clear colorless liquid, should not exist any flocculent precipitation and sludge in visual.

2.The Reagent Blank Value

A) Under A405nm, the reagent blank absorbance should be 0.5000 or less.

B)The determination of reagent blank absorbency rate under A405nm (Δ A/min) should be 0.0030 or less.

3. The Accuracy

A) type of inspection: the comparison test with listed products: correlation coefficient r of 0.975 or higher, in 5 ~ 50 u/L range measurement deviation should be no more than 5 u/L, within the scope of 50 ~ 300 u/L measurement deviation should be 10%more or less.

B) the factory inspection: use quality product were determined and deviation should be no more than plus or minus 10%.

4. Precision

A) repeatability: use the same determination of serum samples repeat 10 times, the measured value of coefficient of variation (CV %) should not more than 5%.

B) tolerance: random difference between the three batch of kits batch should be 10% or less.

5. Linear

5 ~ 300 u/L linear range, the linear correlation coefficient r should be 0.990 or higher, in 5 ~50 u/L (including 50 u/L) determination of the scope of the linear deviation should be no more than 5u/L, in 50 ~ 300 u/L range determination of linear deviation should be no more than 10%.

6.The Sensitivity

concentration of the sample is 165 u/L, the absorbance changed rate is between 0.0050 ~ 0.0800.7

INTERFERENCE

The following analytes were tested up to the levels indicated and found not to interfere:

Hemoglobin: 200 mg/dl
Intralipid: 500 mg/dl
Direct bilirubin: 40 mg/dl
Heparin Sodium: 100 mg/dl
VC: 50mg/dl

SAFETY PRECAUTIONS AND WARNINGS

1. For in vitro diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handing laboratory reagents.
2. Solution R1 contains Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.
3. Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.
4. All specimens used in this test should be considered potentially infectious. Universal Precautions, as they apply at your facility, should be used for handling and disposing of materials during and after testing.

REFERENCES

1. Zielke K. et al. Fucosidsis: diagnosis by serum assay of -L-fucosidase. J. Lab. Clin. Med. 79: 164 (1972)

INDEX OF SYMBOLS

	Manufacture
	Catalogue Number
	Lot number
	Date of manufacture
	Use by(Expiration date)
	For In-Vitro Diagnostic use only
	Stored at 2-8℃
	Attention:See instruction for use
	Authorized Representative in the European Company

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