**α-L-Fucosidase (AFU) Assay Kit**

**Configuration**
The Diazyme AFU reagent is provided in bulk and the following kit configuration:

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Catalog No.</th>
<th>Kit Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Universal</td>
<td>DZ082B-K</td>
<td>R1: 2 x 25 mL</td>
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<tr>
<td></td>
<td></td>
<td>Cal: DZ082B-CAL*</td>
</tr>
</tbody>
</table>

* Calibrator Sold Separately

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

**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% of 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.

**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). AFU control can be purchased separately (Cat. No. DZ082B-CON and DZ082B-C2V)

**Reagent Composition**

| Reagent 1 (R1) | 100 mM Phosphate CNP-AFU Substrate |

**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.

**Specimen Collection and Handling**
Use fresh and non-hemolyzed serum for the 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.
4. Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product. To obtain an MSDS, please contact our customer service department at 858-455-4768.

**Assay Procedure**

| R1: 225 µL |
| Sample: 25 µL |
| 37°C |

0 405nm 3 4 5 6 min A1 A2 A3

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

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

$$ΔA/min = \frac{ΔA_{min} + ΔA_{min+1} + ΔA_{min+2}}{3}$$
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 path-length and other conditions. This can be done experimentally as follows:

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

Application sheets for use of Diazyme AFU Assay on automated clinical chemistry analyzers are available upon request. Please call 858-455-4768 or email: support@diazyme.com.

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

Quality Control
We recommend that each laboratory use AFU controls to validate the performance of AFU reagents. A set of controls is available from Diazyme Laboratories (Cat. No. DZ082B-CON and DZ082B-C2V). 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
  \[ \Delta A/\text{min} = \frac{\Delta A_1/\text{min} + \Delta A_2/\text{min} + \Delta A_3/\text{min}}{3} \]
- Calculate AFU activity (U/L) in the plasma sample by using the formula:
  \[ \text{AFU (U/L)} = \frac{\Delta A/\text{min} \times T_V}{\epsilon \times S_V \times L} = \Delta A/\text{min} \times 1250 \]

\[ \epsilon: \mu \text{molar extinction coefficient of dye} \]
\[ T_V: \text{Total reaction volume (mL)} \]
\[ S_V: \text{Sample volume (mL)} \]
\[ L: \text{Cuvette light path length (1.0cm)} \]

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.

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.

Performance Characteristics

Linearity
The assay is linear from 0-300 U/L (37°C), \( r^2 > 0.99 \)
Precision: Intra assay CV% < 5.1%, and 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