Revised 3 Nov. 2010 rm (Vers. 3.1) **USA: RUO**

Please use only the valid version of the package insert provided with the kit.

1 NAME AND INTENDED USE

This Amylase ELISA is a solid phase enzyme linked immunosorbent assay.

This test provides measurement of human pancreatic amylase (h-p-Amylase) in serum and urine.

This kit is intended for Research Use Only. Not for use in diagnostic procedures.

2 PRINCIPLE OF THE ASSAY

The Amylase ELISA kit is a solid phase enzyme linked immunosorbent assay (ELISA) based on the sandwich principle. The wells are coated with anti-h-p-amylase antibodies. The samples are incubated in the wells with enzyme conjugate which is a mixture of anti-h-p-Amylase antibodies chemically conjugated with horseradish peroxidase. Unbound conjugate is washed off with water. The enzyme conjugate is washed off and the amount of bound peroxidase is proportional to the concentration of the h-p-Amylase present in the samples. Upon addition of the Substrate and Chromogen, the intensity of color developed is proportional to the concentration of h-p-Amylase in the samples.

3 WARNINGS AND PRECAUTIONS

1. This kit is intended for Research Use Only. Not for use in diagnostic procedures.
2. The components in this kit are intended for use as an integral unit. The components from different lots should not be mixed.

4 STORAGE AND STABILITY

1. Store the kit at 2-8°C in a refrigerator. Keep micro-wells sealed in dry bag with desiccants.
2. The unopened reagents are stable until expiration of the kit.
3. TMB Solution should be colorless; if the solution turns blue, it must be replaced.
   Do not expose test reagents to strong light during storage or usage.

5 MATERIALS PROVIDED

1. **Micro-wells strips** (96 wells):
   Anti-h-p-amylase antibodies coated wells, 96 wells.
2. **Sample Diluent** (11 mL):
   1 bottle
3. **Enzyme Conjugate** (11 mL):
   Anti-h-p-amylase antibodies conjugated to horseradish peroxidase.
4. Reference **Standard** Set (0.3 mL each vial)
   The concentrations are 10, 50, 150, 300 and 600 U/L.
5. **Control** (0.3 mL)
   Values as indicated on the vial.

6. **TMB Solution** (11 mL):
   Buffer solution contains hydrogen peroxide and TMB.
7. **Wash Buffer** Concentrate (100X) (10 mL):
   Prepare working washing solution by adding 10 mL washing buffer concentrate into 990 mL distilled water.
8. **Stop Solution**:
   2 N HCl.
9. Well holder: For securing individual wells.

6 MATERIALS REQUIRED BUT NOT PROVIDED

1. Microwell reader.
2. Pipetor with tips for 10 µL, 50 µL & 100 µL.
3. 1 L washing buffer.

7 SAMPLE COLLECTION AND HANDLING

Collect blood aseptically by venipuncture, allow to clot. Separate the serum by centrifugation at room temperature, and store in sterile tubes.
If sera cannot be assayed immediately, they can be stored at 2-8°C for a month or frozen at -20°C for up to 6 months. Avoid repeated freezing and thawing of serum specimens.

8 PREPARATION FOR ASSAY

1. Before beginning the test, bring all samples and reagents to room temperature (24 ± 3°C) and shake gently.
2. Have all reagents and samples ready before the start of the assay. Once the test has begun it must be performed without any interruption to get the most reliable and consistent results.
3. Use new disposable tips for each sample.

9 ASSAY PROCEDURE

1. Secure the desired number of coated wells in holder. Mark data sheet with sample identification.
2. Dispense 10 µL of Standards and samples into appropriate wells.
3. Dispense 100 µL of Enzyme Conjugate into each well.
4. Incubate for 60 minutes at room temperature.
5. Rinse the wells 5 times with washing buffer (300 µL/well/each rinse).
6. Dispense 100 µL of TMB Solution into each well including blank well.
7. Incubate for 30 minutes at room temperature.
8. Stop reaction by adding 50 µL of Stop solution to each well and read OD at 450 nm with microwell reader.

10 PROCEDURAL NOTE

1. It is very important to wash the microwells thoroughly and remove the last droplets of washing buffer to achieve the best results.
2. Pipet all reagents and samples into the bottom of well. Avoid scratching the well. Vortex-mixing or shaking of wells is not required.
3. Absorbance is the function of the time and temperature of incubations. It is recommended to have all reagents and samples caps removed. All needed wells secured in holder and assigned. This will ensure the equal elapsed time for each pipetting without interruption.
4. For the same reason, the size of the assay run should be limited. It is suggested to run no more than 20 samples with a set of Reference Standards in duplicate.
5. If in an assay, a serum specimen has been found to contain greater than 1000 U/L of h-p-amylase, the sample must be further diluted with sample diluent and re-assayed as described in Assay Procedure.

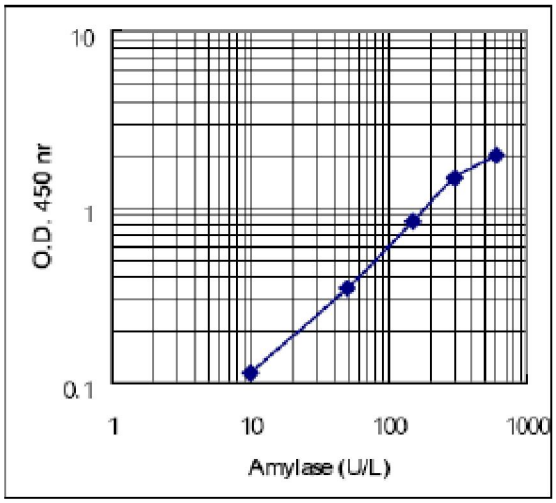
11 CALCULATION OF RESULTS

Any microwell reader capable of determining absorbance at 450 nm may be used. The h-p-amylase value of the sample is obtained as follows:

1. Plot the concentration (X) of each Reference Standards against its absorbance (Y) on graph paper.
2. Obtain the h-p-amylase values of samples by reference to the standard curve. The table and the figure are the example.

(NOTE: The data is for demonstration purpose only and must not be used in place of data generated for each assay.)

Well No.	Description (U/L)	Absorbance (450 nm)	Value from Std. Curve
A1 B1	0 U/L	0.000 0.000	
A2 B2	10 U/L	0.116 0.112	
A3 B3	50 U/L	0.276 0.262	
A4 B4	150 U/L	0.828 0.848	
A5 B5	300 U/L	1.469 1.499	
A6 B6	600 U/L	1.971 2.035	
A7 B7	Sample A (serum)	0.602 0.591	200



12 REFERENCES / LITERATURE

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