alpha Amylase Activity Assay Kit (Colorimetric)

alpha Amylase Activity Assay Kit (Colorimetric) can be used to measure alpha Amylase in serum, saliva and urine samples.

Catalog number: ARG82136

Package: 100 tests

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Alpha-amylase, (α-amylase) is an enzyme EC 3.2.1.1 that hydrolyses alpha bonds of large, alpha-linked polysaccharides, such as starch and glycogen, yielding glucose and maltose. It is the major form of amylase found in humans and other mammals. It is also present in seeds containing starch as a food reserve, and is secreted by many fungi.

The test for amylase is easier to perform than that for lipase, making it the primary test used to detect and monitor pancreatitis. Medical laboratories will usually measure either pancreatic amylase or total amylase. If only pancreatic amylase is measured, an increase will not be noted with mumps or other salivary gland trauma.

However, because of the small amount present, timing is critical when sampling blood for this measurement. Blood should be taken soon after a bout of pancreatitis pain, otherwise it is excreted rapidly by the kidneys.

Salivary α-amylase has been used as a biomarker for stress and as a surrogate marker of sympathetic nervous system (SNS) activity that does not require a blood draw.

Increased plasma levels in humans are found in: Salivary trauma (including anaesthetic intubation); Mumps – due to inflammation of the salivary glands; Pancreatitis – because of damage to the cells that produce amylase; renal failure – due to reduced excretion

Total amylase readings of over 10 times the upper limit of normal (ULN) are suggestive of pancreatitis. Five to 10 times the ULN may indicate ileus or duodenal disease or renal failure, and lower elevations are commonly found in

salivary gland disease. [Wikipedia: alpha Amylase]

PRINCIPLE OF THE ASSAY

This alpha Amylase Activity Assay Kit provides a Simple, direct and automation-ready procedures for measuring alpha Amylase concentrations in samples. This alpha Amylase Activity Assay Kit involves two step reactions to measuring alpha Amylase activity. At first, alpha amylase in the sample hydrolyzes starch and the products are rapidly converted to glucose by alpha glucosidase and then hydrogen peroxide (H2O2) is produced by glucose oxidase. Then the hydrogen peroxide (H2O2) concentration is determined with a colorimetric reagent and be detected at OD 585 nm.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
Assay Buffer	20 ml (Ready to use)	-20°C
Detection Reagent	20 ml (Ready to use)	-20°C
Glucose Standard (300 mg/dL)	1 ml	-20°C
Substrate	120 µl	-20°C
Enzyme A	120 µl	-20°C
Enzyme B	120 µl	-20°C

The kit is shipped on ice. Store all component at -20°C. Shelf life of 6 months after receipt.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 585 nm.
- Flat bottomed 96-well microplate
- Pipettes and pipette tips
- Deionized or distilled water
- 1.5-mL centrifuge tubes
- (Optional) membrane filters (e.g. Microcon YM-10 from Millipore)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- It is recommended that the standards and samples be assayed in duplicates.
- Keep thawed Enzyme Mix in a refrigerator or on ice before use.
- All reagents should be warmed to room temperature before use.
- The substrate may have precipitates. Prior to use, vortex tube to dissolve precipitates; gentle swirl the Detection Reagent bottle.
- Briefly spin down the reagents before use.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g at 2-8°C. Collect serum and assay immediately or aliquot & store samples at ≤-20°C for up to one month. Avoid repeated freeze-thaw cycles. It is suggested to assay the samples freshly after collection.

Note:

1. Ascorbic acid, heparin, EDTA, EGTA, citrate, SDS, Tris (> 8mM) and ethanol (>0.4%) interfere and should be avoided in sample preparation.
2. For samples known to contain glucose, use a membrane filter (e.g. Microcon YM-10 from Millipore) to remove glucose:
    1). Load 50 µL sample in a Microcon YM-10 (10 kDa cutoff) and add 500 µL Assay Buffer.
    2). Centrifuge at 14000 rpm for 30 min, check level of sample, ideally the sample level will be less than 50 µL. Add 500 µL Assay Buffer again and repeat the centrifugation.
    3). Measure the volume of final sample with a pipetman and calculate dilution factor n = final sample volume/50 µL.
3. It is suggested to perform a pilot test with samples at various dilutions. Recommended dilution: serum 50-fold, saliva 2,000-fold in Assay Buffer prior to assay.
4. Samples should be clear and free of particles or precipitates. Avoid using

haemolytic, icteric or lipaemic samples.

REAGENT PREPARATION

- Standard:

  - Dilute 10 µl of 300 mg/dL Glucose standard stock solutions with 406 µl of Assay Buffer to yield a working standard solution concentration of 400 µM.

- Working Reagent: *Prepare before use,* prepare enough Working Reagent to be used.

  For each well:

  40 µl of Assay Buffer,

  0.5 µl of Substrate,

  1 µl of Enzyme A,

  1 µl of Enzyme B.

  Mix well. Add 40 µL of Working Reagent quickly to each well. Tap the plate to mix.

- Samples:

  - If the calculated activity is higher than 50 U/L, dilute sample in Assay Buffer and repeat the assay again and the calculated concentration must be further converted by the appropriate dilution factor.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use, each vial should be mixed thoroughly without foaming and briefly centrifuge tubes prior to use.

1. Add 10 µl of Assay Buffer in one well of the clear flat bottomed 96-well microplate as background control (Blank) well.
2. Add 10 µl of each sample and standard (400 µM glucose) into the corresponding wells.
3. Prepare Working Reagent as the instruction in REAGENT PREPARATION section. Add 40 µl of Working Reagent quickly after preparation into each well. Gently tap the plate to ensure thorough mixing.
4. Incubate the plate at room temperature (25°C) for 15 min.
5. Add 150 µl of Detection Reagent to each well. Tap the plate to mix it well immediately.
6. Incubate the plate at room temperature (25°C) for 20 min.
7. Read O.D. with a microplate reader at 585 nm (540-610nm) immediately.

Summary of Assay Procedure

Reagent	Sample	Standard	Blank
Sample	10 µl	-	-
Standard	-	10 µl	-
Assay buffer	-	-	10 µl
Working Reagent	40 µl	40 µl	40 µl
Gently tap plate to mix thoroughly. Incubate the plate **at RT for 15 min**			
Detection Reagent	150 µl	150 µl	150 µl
Gently tap plate to mix thoroughly. Incubate the plate **at RT for 20 min**			
Read O.D. with a microplate reader at 585 nm (540-610nm) immediately			

CALCULATION OF RESULTS

1. Calculate the alpha Amylase activity of the Samples as follows:

   alpha Amylase activity (U/L) =

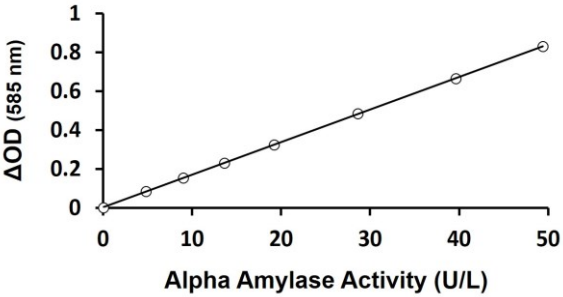
   $n \times [(OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}] \times [400/ t (min)]$

   Note:

   - The OD_{Sample}, $OD_{Standard}$ and OD_{Blank} are optical density values of the sample, 400 µM glucose standard and blank.
   - t is the incubation time. t = 15 min in the standard protocol.
   - n is the dilution factor (n = 50 for serum, 2000 for saliva).

2. One unit of enzyme catalyzes the production of 1 µmole of glucose per min under the assay conditions.
3. If the calculated activity is higher than 50 U/L, sample should be further diluted by Assay Buffer and repeat assay. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor.

EXAMPLE OF ASSAY



QUALITY ASSURANCE

Sensitivity

Use as little as 10 µL samples. Linear detection range 0.3 to 50 U/L of alpha Amylase in 96-well plate.

The minimum detectable dose (MDD) of alpha Amylase was: 0.3 U/L.