Human alpha amylase ELISA Kit

Enzyme Immunoassay for the quantification of Human alpha amylase in saliva.

Catalog number: ARG82783

Package: 96 wells

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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 shorter chains thereof, dextrins, 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. It is a member of glycoside hydrolase family 13.

Although found in many tissues, amylase is most prominent in pancreatic juice and saliva, each of which has its own isoform of human α-amylase. They behave differently on isoelectric focusing, and can also be separated in testing by using specific monoclonal antibodies. In humans, all amylase isoforms link to chromosome 1p21. [Provided by Wikipedia: Alpha-amylase]

PRINCIPLE OF THE ASSAY

This assay employs the competitive quantitative enzyme immunoassay technique. A highly specific antibody for α-amylase has been pre-coated onto a microplate. α-amylase containing samples, Controls or Standards and α-amylase-HRP conjugate are given into the wells of the microtiter plate. Simultaneous add Antibody Conjugate into the plate. Enzyme labeled and free α-amylase compete for the antibody binding sites. After incubation at room temperature, the wells are washed with diluted Wash Buffer to remove unbound material. Then TMB substrate is added to the wells and color develops in inversely proportion to the amount of α-amylase present in the samples. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450 nm. The concentration of α-amylase in the samples is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C. Do not freeze. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated microplate	8 X 12 strips	4°C
Standards A to F (0, 10, 30, 80, 200, 500 U/mL)	1 mL each (ready to use)	4°C
Control 1 & 2	1 mL each (ready to use)	4°C
Antibody Conjugate	12 mL (ready to use)	4°C
Diluent Buffer	100 mL (ready to use)	4°C
Alpha Amylase-HRP Conjugate	12 mL (ready to use)	4°C
10X Wash Buffer	100 mL	4°C
TMB substrate	12 mL (ready to use)	4°C (protect from light)
STOP solution	12 mL (ready to use)	4°C
Protective Foil	3 pieces	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Mixer or Ultra-Turrax
- Microplate shaker
- Pipettes and pipette tips
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (18-25°C) approx. 30 minutes before use.
- Remove the number of strips required and return unused strips to the pack and reseal.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not

become completely dry during the assay.

- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Saliva:

1. Collect saliva sample with a centrifuge glass tube (contain a plastic straw).
2. Centrifuge the sample at 3000 rpm for 15 minutes.
3. Store at-20°C for at least 1 hour.
4. Centrifuge again at 3000 rpm for 15 minutes.
5. Use supernatant for the assay.

Note:

1. Samples containing sodium azide should not be used in the assay.
2. Specimens should be capped and may be stored for up to 14 days at 2-8°C prior to assaying. Diluted sample should be incubated within one working day.
3. Sample are diluted with Diluent Buffer at ratio 1:200.
   E.g. 5 µL sample to 1.0 mL Diluent Buffer and mix well by vortexing (sample pipettes are not suitable for mixing).
4. Standards and Controls are pre-diluted and ready for use, do not dilute them.

REAGENT PREPARATION

- **1X Wash Buffer:** Dilute 10X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 50 mL of 10X Wash Buffer into 450 mL of distilled water to a final volume of 500 mL) The 1X Wash Buffer is stable for up to 4 weeks at 2-8°C.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 18-25°C) approx. 30 minutes before use. Standards and samples should be assayed in duplicates.

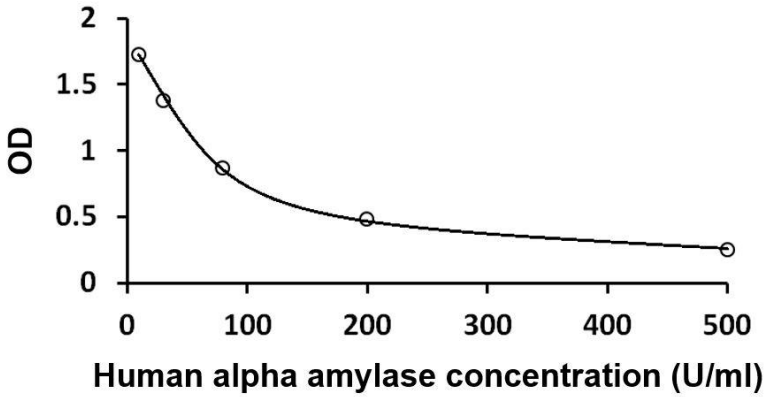
1. Add **20 µL** of **Standards, Controls and diluted samples** into the appropriate wells of the Antibody Coated Microplate.
2. Add **100 µL** of **Alpha Amylase-HRP Conjugate** and **100 µL** of **Antibody Conjugate** into all wells.
3. Incubate at **RT** for **60 minutes** on a microplate shaker.
4. Aspirate each well and wash, repeating the process 2 times for a total **3 washes.** Wash by filling each well with **1× Wash Buffer (300 µL)** using a squirt bottle, manifold dispenser, or autowasher. Leave the **1X Wash Buffer** in each well for **30 to 60 seconds** per washing cycle. Then complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add **100 µL** of **TMB Substrate** to each well, including the blank wells. Incubate in the dark for **15 minutes** at **RT** on a microplate shaker.
6. Immediately Add **100 µL** of **Stop Solution** to each well, including the blank

wells. The color of the solution should change from blue to yellow.

7. Read the OD with a microplate reader at 450 nm immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance within 30 minutes after adding the stop solution.

EXAMPLE OF TYPICAL STANDARD VALUES

The following figures demonstrate typical results with the Human alpha amylase ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of Controls, standards and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
4. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
5. For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another the sample should be retested.
6. If the extinction of a patient sample lies above the value of Standard F (500 U/mL), the result should be reported as "> 500 U/mL".

QUALITY ASSURANCE

Sensitivity

The sensitivity of the Human alpha amylase ELISA kit is 3.6 U/mL.

Specificity

Substance	Cross Reactivity (%)
Alpha amylase in human saliva	100
Porcine pancreatic alpha amylase	< 0.23
Alpha amylase from Bacillus sp.	< 0.01

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 3.6-5.5% and CV value of inter-assay precision was 4.2-9.6%.