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## Datasheet for ABIN612663

### Amylase ELISA Kit

#### Overview

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|--------------------------|----------------|
| Quantity:                | 96 tests       |
| Target:                  | Amylase (AMY)  |
| Reactivity:              | Human          |
| Method Type:             | Sandwich ELISA |
| Minimum Detection Limit: | 0.3 mU/mL      |
| Application:             | ELISA          |

#### Product Details

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| Purpose:           | The AssayMax Human Amylase ELISA kit is designed for detection of human Amylase in plasma, serum, urine, milk, saliva, and cell culture supernatants   |
| Brand:             | AssayMax   |
| Sample Type:       | Plasma, Cell Culture Supernatant   |
| Analytical Method: | Quantitative   |
| Detection Method:  | Colorimetric   |
| Components:        | <p>Amylase Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Amylase. 1 Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.</p> <p>Amylase Standard: Human Amylase in a buffered protein base (160 mU, lyophilized).</p> <p>Biotinylated Amylase Antibody (100x): A 100-fold concentrated biotinylated polyclonal antibody against FVII (80µl). Mix Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml). Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 ml, 2</p> |

## Product Details

bottles). Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (80µl).  
Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml). Stop Solution: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Material not included: Microplate reader capable of measuring absorbance at 450 nm. Pipettes (1-20 µL, 20-200 µL, 200-1000µL and multiple channel). Deionized or distilled reagent grade water

## Target Details

Target: Amylase (AMY)

Alternative Name: Amylase ([AMY Products](#))

Background: Human amylase is a secreted enzyme that is present in saliva and pancreatic secretions in the form of alpha-amylase with 496 amino acids and 56 kDa (1-3). Salivary alpha-amylase catalyses the hydrolysis of 1,4-alpha-glycosidic bonds of starch into disaccharide maltose, trisaccharide maltotriose, and small dextrans. Pancreatic alpha-amylase continues the hydrolysis of starch into disaccharides and trisaccharides which are converted by alpha-glucosidases to absorbable glucose, fructose, and galactose in the small intestine. The serum amylase concentration is increased in acute pancreatitis, and ovarian tumors (4-6). By retardation of carbohydrate digestion, the amylase inhibitor has anti-obesity and anti-diabetes effects and can control postprandial hyperglycemia in type 2 diabetes (7-8). Salivary alpha-amylase has been proposed as a stress biomarker in autonomic/sympathetic nervous system.

## Application Details

Sample Volume: 50 µL

Assay Time: < 4 h

Plate: Pre-coated

Protocol: This assay employs a quantitative sandwich enzyme immunoassay technique that measures Amylase in less than 4 hours. A polyclonal antibody specific for Amylase has been pre-coated onto a 96-well microplate with removable strips. Amylase in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for Amylase, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

## Application Details

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**Reagent Preparation:** Freshly dilute all reagents and bring all reagents to room temperature before use. Mlx Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Mlx Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8°C. Standard Curve: Reconstitute the 160 mU of human FVII Standard with 4 ml of Mlx Diluent to generate a stock solution of 40 mU/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the stock solution (40 mU/ml) twofold with equal volume of Mlx Diluent to produce 20, 10, 5, 2.5, 1.25, 0.625 and 0.313 mU/ml. Mlx Diluent serves as the zero standard (0 mU/ml). Any remaining solution should be frozen at -20°C. Standard Point Dilution [Amylase] (mU/ml) P1 1 part Standard (40 mU/ml) + 1 part Mlx Diluent 20.00 P2 1 part P1 + 1 part Mlx Diluent 10.00 P3 1 part P2 + 1 part Mlx Diluent 5.000 P4 1 part P3 + 1 part Mlx Diluent 2.500 P5 1 part P4 + 1 part Mlx Diluent 1.250 P6 1 part P5 + 1 part Mlx Diluent 0.625 P7 1 part P6 + 1 part Mlx Diluent 0.313 P8 Mlx Diluent 0.000 Biotinylated Amylase Antibody (100x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with Mlx Diluent. Any remaining solution should be frozen at -20°C. Wash Buffer Concentrate (20x): Dilute the Wash Buffer Concentrate 1:20 with reagent grade water. SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with Mlx Diluent. Any remaining solution should be frozen at -20°C.

**Sample Collection:** Plasma: Collect plasma using 3.8% sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Dilute samples 1:20 into Mlx Diluent. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA can also be used as anticoagulant). Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. Dilute samples 1:20 into Mlx Diluent. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Cell Culture Supernatants: Collect cell culture media and centrifuge at 2000 x g for 10 minutes at 40C to remove debris. The samples can be stored at -20°C or below. Avoid repeated freeze-thaw cycles. Saliva: Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes and assay. Dilute samples 1:15000 into Mlx Diluent. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Urine: Collect urine using sample pot. Centrifuge samples at 600 x g for 10 minutes. Dilute samples 1:100 into Mlx Diluent Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. 2 Milk: Collect milk using sample tube. Centrifuge samples at 600 x g for 10 minutes. Dilute samples 1:400 into Mlx Diluent Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

## Application Details

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**Assay Procedure:** Prepare all reagents, working standards and samples as instructed. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator. Add 50  $\mu$ L of Standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition. Wash five times with 200  $\mu$ L of Wash Buffer manually. Invert the plate each time and decant the contents, hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300  $\mu$ L of Wash Buffer and then invert the plate, decant the contents, hit it 4-5 times on absorbent paper towel to completely remove the liquid. Add 50  $\mu$ L of Biotinylated Amylase Antibody to each well and incubate for one hour. Wash the microplate as described above. Add 50  $\mu$ L of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance. Wash the microplate as described above. Add 50  $\mu$ L of Chromogen Substrate per well and incubate for approximately 10 minutes or till the optimal blue color density develop. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip. Add 50  $\mu$ L of Stop Solution to each well. The color will change from blue to yellow. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

**Calculation of Results:** Calculate the mean value of the triplicate readings for each standard and sample. To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor. Standard Curve The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.

**Assay Precision:** Intra-assay and inter-assay coefficients of variation were 5.2 % and 7.3 % respectively.

**Restrictions:** For Research Use only

## Handling

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**Handling Advice:** Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated- antibody, and SP conjugate) as instructed, prior to running the assay. Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user

## Handling

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should determine the optimal dilution factor. Spin down the SP conjugate vial and the biotinylated-antibody vial before opening and using contents. The kit should not be used beyond the expiration date.

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| Storage: | 4 °C/-20 °C |
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| Storage Comment: | Store components of the kit at 2-8°C or -20°C upon arrival up to the expiration date. Store SP Conjugate and Biotinylated Antibody at -20°C Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator. Diluent (1x) may be stored for up to 1 month at 2-8°C. Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent |
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