AssayLite™

Human Amylase Fluorescent Immunoassay Kit

(Red Fluorescent Probe)

Assaypro LLC
3400 Harry S Truman Blvd
St. Charles, MO 63301
T (636) 447-9175
F (636) 395-7419
www.assaypro.com

This product is manufactured under patented technology by Assaypro LLC

US Patent No. 9,945,847

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank you for choosing Assaypro.

Table of Contents

1 Assay Summary
2 Assay Mechanism
3 Assay Template
4 Introduction
4 Principle of the Assay
4 Caution and Warning
5 Reagents
5 Storage Conditions
5 Other Supplies Required
6 Sample Collection, Preparation, and Storage
7 Reagent Preparation
8 Assay Procedure
9 Data Analysis
9 Standard Curve
10 Performance Characteristics
11 Troubleshooting
12 References

Assay Summary

Step 1. Add 100 µl of Standard/Sample per well.
Incubate 2 hours at 37°C.

Step 2. Wash, then add 100 µl of Fluorescent Probe per well.
Incubate 1 hour at 37°C.

Step 3. Wash, then add 50 µl of Stabilizing Solution per well.

Step 4. Read at EX 485/20 nm, EM 575/15 nm

Symbol Key

Consult instructions for use.

Assay Mechanism

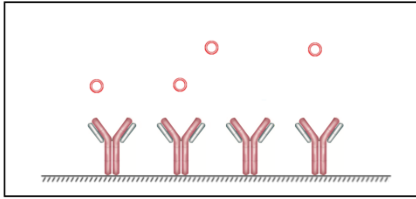


Figure 1. Standards and samples are added to wells and incubated.

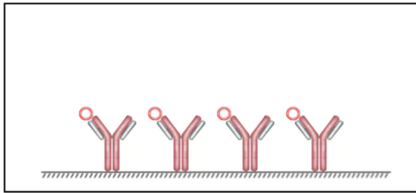


Figure 2. The Antigen A in standards and samples binds to the immobilized Antibody A. All unbound materials are washed away.

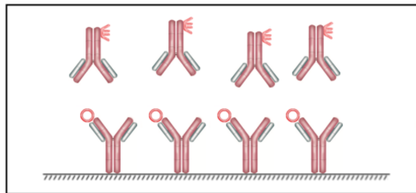


Figure 3. The Antibody A Fluorescent Probe is added to wells and binds to the Antigen A.

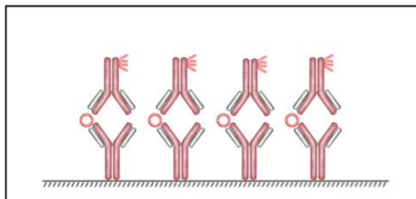
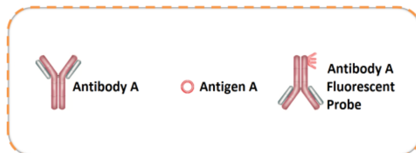


Figure 4. The microplate is washed and the endpoint fluorescence is measured. The fluorescence intensity is proportional to the concentration of Antigen A in the standard or samples.



Assay Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

AssayLite™ Human Amylase Fluorescent Immunoassay Kit

Catalog No. FEA6501

Sample insert for reference use only

Introduction

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-α-glycosidic bonds of starch into disaccharide maltose, trisaccharide maltotriose, and small dextrins. 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 (9).

Principle of the Assay

The AssayLite™ Human Amylase Fluorescent Immunoassay Kit employs a **quantitative sandwich fluorescent probe technique** that measures amylase in human **plasma, serum, urine, saliva, and milk samples** in approximately 3 hours. A polyclonal antibody specific for human amylase has been pre-coated onto a 96-well opaque microplate with removable strips. Amylase in standards and samples is sandwiched by the immobilized antibody and a polyclonal antibody specific for human amylase conjugated to a fluorescent probe. All unbound material is washed away before the endpoint fluorescence is measured.

Caution and Warning

- This product is for **Research Use Only** and is not intended for use in diagnostic procedures.
- Prepare all reagents (diluent buffer, wash buffer, standard, and fluorescent probe), 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 insert. However, the user should determine the optimal dilution factor.
- The kit should not be used beyond the expiration date.

- Avoid direct light exposure to the assay.
- Store fluorescent probe in a dark place. Do not freeze.

Reagents

- **Human Amylase Microplate:** A 96-well opaque polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human amylase.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive aluminum sealing tapes that can be cut to fit the format of the individual assay.
- **Human Amylase Standard:** Human amylase in a buffered protein base (10 mIU, lyophilized, 3 vials).
- **Red Fluorescent Human Amylase Probe (2x):** Lyophilized, 5 vials.
- **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).
- **Stabilizing Solution (1x)**: A solution to stabilize the fluorescent component (8 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store Microplate, Diluent Concentrate (10x), Stabilizing Solution, and Wash Buffer at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccants and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.
- Store Fluorescent Probe in a dark place at 2-8°C. Do not freeze.

Other Supplies Required

- Pipettes (1-20 μl, 20-200 μl, 200-1000 μl, and multiple channel)
- Deionized or distilled reagent grade water
- Incubator (37°C)
- Fluorescent Microplate Reader

Sample Collection, Preparation, and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes and collect plasma. A 40-fold sample dilution is suggested into MIX Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes and remove serum. A 40-fold sample dilution is suggested into MIX Diluent; however, user should determine optimal dilution factor depending on application needs. 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 800 x *g* for 10 minutes. A 5-fold sample dilution is suggested into MIX Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Saliva:** Collect saliva using samples tube. Centrifuge samples at 800 x *g* for 10 minutes. A 40000-fold sample dilution is suggested into MIX Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Milk:** Collect milk using sample tube. Centrifuge samples at 800 x *g* for 10 minutes. A 400-fold sample dilution is suggested into MIX Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Applicable samples may also include biofluids, cell culture, and tissue homogenates. If necessary, user should determine optimal dilution factor depending on application needs.

Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater *(for reference only; please follow the insert for specific dilution suggested)*	
100x	**10000x**
A) 4 µl sample: 396 µl buffer (100x) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl.*
1000x	**100000x**
A) 4 µl sample : 396 µl buffer (100x) B) 24 µl of A : 216 µl buffer (10x) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) C) 24 µl of B : 216 µl buffer (10x) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl.*

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **MIX Diluent Concentrate (10x)**: Dilute the MIX Diluent Concentrate 10-fold with reagent grade water to produce a 1x solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved. Store for up to 30 days at 2-8°C.
- **Human Amylase Standard:** Reconstitute the Human Amylase Standard (10 mIU) with 0.5 ml of MIX Diluent to generate a 20 mIU/ml standard stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (20 mIU/ml) 2-fold with equal volume of MIX Diluent to produce 10, 5, 2.5, 1.25, 0.625, and 0.313 mIU/ml solutions. MIX Diluent serves as the zero standard (0 mIU/ml). Aliquot remaining stock solution to limit repeated freeze-thaw cycles. This solution should be stored at -20°C and **used within 5 days**.

Standard Point	Dilution	[Amylase] (mIU/ml)
P1	1 part Standard (20 mIU/ml)	20
P2	1 part P1 + 1 part MIX Diluent	10
P3	1 part P2 + 1 part MIX Diluent	5
P4	1 part P3 + 1 part MIX Diluent	2.5
P5	1 part P4 + 1 part MIX Diluent	1.25
P6	1 part P5 + 1 part MIX Diluent	0.625
P7	1 part P6 + 1 part MIX Diluent	0.313
P8	MIX Diluent	0

- **Red Fluorescent Human Amylase Probe (2x):** Reconstitute the fluorescent probe with 1 ml of MIX Diluent to generate a stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to dilution. From the stock solution, dilute 2-fold with MIX Diluent to produce a 1x working solution. Any remaining stock solution should be stored at 2-8°C and **used within 10 days**. Do not freeze.
- **Wash Buffer Concentrate (20x):** Dilute the Wash Buffer Concentrate 20-fold with reagent grade water to produce a 1x solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is incubated at 37°C.
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 100 µl of Human Amylase Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash the microplate manually or automatically using a microplate washer. Invert the plate and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If washing manually, wash five times with 200 µl of Wash Buffer per well. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a microplate washer, wash six times with 300 µl of Wash Buffer per well; invert the plate and hit 4-5 times on absorbent material to completely remove the liquid.

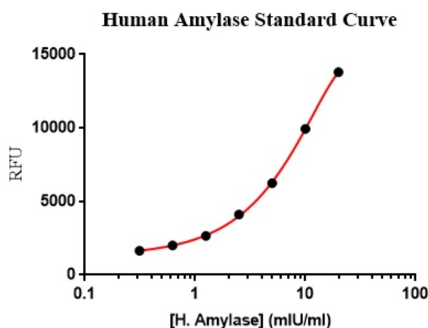
- Add 100 µl of Red Fluorescent Human Amylase Probe to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 1 hour.
- Wash the microplate as described above.
- Immediately add 50 µl of Stabilizing Solution to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed.
- Read the endpoint fluorescence on a microplate reader at an excitation wavelength of 485/20 nm and emission wavelength of 575/15 nm.
- For the Synergy H1F, a gain of 75 is suggested; however, the user should determine the optional gain/amplification.

Data Analysis

- Calculate the mean value of the duplicate or 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 emitted fluorescence 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.



Note: Standard curve reading with Biotek® Synergy H1F
$R^2 = 0.998$

Performance Characteristics

Reader	Standard Point	mIU/ml	Human Amylase Average Red RFU
BioTek – Synergy H1F	P1	20	13792
	P2	10	9934
	P3	5	6245
	P4	2.5	4101
	P5	1.25	2656
	P6	0.625	2002
	P7	0.313	1646
	P8	0	1176

- The minimum detectable dose of human amylase as calculated by 2SD from the mean of a zero standard was established to be 0.22 mIU/ml.
- Intra-assay precision was determined by testing three plasma samples twenty times in one assay.
- Intra-assay and inter-assay coefficients of variation were 4.3% and 5.8%, respectively.

Reference Value

- Normal human amylase plasma and serum levels range from 0 – 140 mIU/ml.
- Plasma and serum samples from healthy adults were tested (n=40). On average, human amylase level was 62 mIU/ml.

Sample	n	Average Value (mIU/ml)
Pooled Normal Plasma	10	53
Normal Plasma	20	63
Pooled Normal Serum	10	70

Recovery

Standard Added Value	0.625 – 5 mIU/ml
Recovery %	85 - 112%
Average Recovery %	98.5%

Linearity

- Plasma and serum samples were serially diluted to test for linearity.

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
20x	89%	90%
40x	96%	97%
80x	110%	104%

Cross-Reactivity

Species	Cross-Reactivity (%)
Bovine	None
Canine	40%
Monkey	80%
Mouse	5%
Rabbit	None
Rat	None
Swine	15%

Troubleshooting

Issue	Causes	Course of Action
Low Precision	Use of improper components	• Check the expiration date listed before use. • Do not interchange components from different lots.
	Improper wash step	• Check that the correct wash buffer is being used. • Check that all wells are empty after aspiration. • Check that the microplate washer is dispensing properly. • If washing by pipette, check for proper pipetting technique.
	Splashing of reagents while loading wells	• Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the Standard and Fluorescent Probe after reconstitution. • Thoroughly mix dilutions.
	Improperly sealed microplate	• Check the microplate pouch for proper sealing. • Check that the microplate pouch has no punctures. • Check that three desiccants are inside the microplate pouch prior to sealing.
Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	• Each step of the procedure should be performed uninterrupted.
	Omission of step	• Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	• Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	• Check pipette calibration. • Check pipette for proper performance.
	Wash step was skipped	• Consult the provided procedure for all wash steps.

	Improper wash buffer	• Check that the correct wash buffer is being used.
	Improper reagent preparation	• Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	• Consult the provided procedure for correct incubation time.
	Prolonged exposure of assay or Fluorescent Probe to light	• Overexposure can affect the stability of the Fluorescent Probe, store in a dark location. • Cover and cap all reagents when not in use. • Cover assay with aluminum sealing film immediately after loading.
	Contamination of reagents	• A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporated	• Verify that the aluminum sealing film is firmly in place before placing the assay in the incubator.
	Used filters with an overlapping bandpass	• As an example, do not use a filter combination of 620/20 EX and 660/40 EM, use a 660/20 filter instead.
Deficient Standard Curve Fit	Improper pipetting	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the Standard and Fluorescent Probe after reconstitution. • Thoroughly mix dilutions.

References

(1) Nishide T *et al*. (1986) *Gene*. 41(2-3):299-304.
(2) Wise RJ *et al*. (1984) *Mol Biol Med*. 2(5):307-322.
(3) Horii A *et al*. (1987) *Gene*. 60(1):57-64.
(4) Banks PA *et al*. (2006) *Am J Gastroenterol*. 101(10):2379-2400.
(5) Shikata J *et al*. (1981) *Int Surg*. 66(4):319-324.
(6) Van Kley H *et al*. (1981) *Cancer*. 48(6):1444-1449.
(7) Golay A *et al*. (1991) *Am J Clin Nutr*. 53(1):61-65.
(8) Tsujita T *et al*. (2008) *J Nutr Sci Vitaminol (Tokyo)*. 54(1):82-88.
(9) Granger DA *et al*. (2007) *Ann NY Acad Sci*. 1098:122-44.

Version 1.0R