# Stanbio Alpha-Amylase LiquiColor® Procedure No. 2970

For the Quantitative Determination of Amylase in Serum and Urine

## Summary and Principle

Alpha-amylase is an enzyme found in bacteria and animal tissues that catalyzes the hydrolytic cleavage of starch and glycogen. Serum amylase activity determinations are of clinical interest in the diagnosis of pancreatic function.[1]

The enzymatic procedure presented is based on modifications of Wallenfels, using as substrate p-Nitrophenyl-D-maltoheptaoside (PNPG7) with the terminal glucose blocked to reduce spontaneous degradation of the substrate by glucosidase and glycoamylase.[2] The test is performed in a kinetic mode with a very short lag time and offers much greater stability than previous amylase methodologies.

Amylase hydrolyzes p-Nitrophenyl D-maltoheptaoside (PNPG7) to p-Nitrophenyl-maltotriose (PNPG3) and maltotetraose. Glucoamylase hydrolyzes PNPG3 to p-Nitrophenylglycoside (PNPG1) and glucose. Then PNPG1 is hydrolyzed by glucosidase to glucose and p-Nitrophenol, which produces a yellow color. The rate of increase in absorbance is measured at 405 nm and is proportional to the amylase activity in the sample.

## Reagent

**Amylase LiquiColor Reagent, Cat. No. 2971**

| | | |
|---|---|---|
| HEPES Buffer, pH 7.1 ± 0.1 | 0.1 | mol/L |
| Glucosidase (microbial) | > 6 | KU/L |
| Sodium Chloride | 62.5 | mmol/L |
| Magnesium Chloride | 12.5 | mmol/L |
| EPS-G7 (PNPG7) | > 8 | mmol/L |

**Precautions:** *For In Vitro Diagnostic Use.*
DO NOT PIPETTE REAGENT BY MOUTH to avoid contamination with salivary amylase.

**Reagent Preparation:** Amylase liquid reagents are supplied ready-to-use.

**Reagent Storage and Stability:** Reagent is stable until the expiration date on their respective labels, when properly stored at 2-8°C and protected from light. Discard reagent if it appears cloudy or contains particulate matter.

## Materials Required But Not Provided

Spectrophotometer capable of absorbance readings at 405 nm (400-420 nm)
Constant temperature block or bath, 37°C, or temp. controlled cuvet well
Accurate pipetting devices
Test tubes
Interval timer

## Specimen Collection and Preparation

Unhemolyzed serum is the specimen of choice. Plasma from heparin tubes may be used. Other anticoagulants, such as Citrate and EDTA, bind calcium, an ion needed for amylase activity. Therefore, plasma with any anticoagulant other than heparin should not be used.

Urine specimens should be adjusted to a pH of 7.0 and kept refrigerated until assayed.

**Sample Stability:** Amylase in serum and urine is reported stable for one week at room temperature (15-30°C) and for several months when stored refrigerated and protected against evaporation and bacterial contamination.[3]

**Interfering Substances:** A number of drugs and substances affect the determination of amylase. Young et al have published a comprehensive list of such substances.[4]

## Automated Analyzers

**Parameters:**

| | |
|---|---|
| Wavelength | 405 nm |
| Reaction Type | Kinetic |
| Reaction Direction | Increasing |
| Reaction Temperature | 37°C |
| Sample/Reagent Ratio | 1:40 |
| Lag Time | 30 Seconds |
| Read Time | 30 Seconds |
| Factor | 4824 |
| Low Normal | 25 U/L |
| High Normal | 125 U/L |
| Linearity | 2,000 U/L |
| Cuvet Lightpath | 1 cm |

Above parameters should be employed in programming automated analyzers for Amylase. Consult your instrument manual for programming instructions.

## Manual Procedure

1. For each sample, add 1.0 mL reagent to cuvet or test tube and prewarm at 37°C for at least 3 minutes.
2. Zero spectrophotometer with water at 405 nm.
3. Add 0.025 mL (25 µL) serum to its respective tube and read immediately.
4. Record increase in absorbance at 30 second intervals for 2 minutes.
5. Determine the mean absorbance difference per minute (ΔAbs./min.)
6. Multiply the ΔAbs./min. by 4824 to obtain the result in U/L.

**NOTE**: If cuvet is not temperature controlled, incubate samples at 37°C between readings.

**Quality Control:** Two levels of control material with known amylase levels determined by this method should be analyzed each day of testing.

## Results

Values are derived based on the millimolar absorptivity of p-Nitrophenol which is 8.5 at 405 nm under the test conditions described.

$$U/L = \frac{\Delta Abs./min. \times Total\ volume \times 1000\ (U/mL\ to\ U/L)}{Absorptivity \times Sample\ volume \times Light\ path}$$

$$U/L = \frac{\Delta Abs./min. \times 1.025 \times 1000}{8.5 \times 0.025 \times 1.0}$$

$$U/L = \Delta Abs./min. \times 4824$$

## Expected Values[5]

| | | |
|---|---|---|
| Normal Range | Serum | 25 - 125 U/L |
| | Urine | 1 - 17 U/Hour |

The range should serve only as a guideline. Since the expected values are affected by age, sex, diet and geographical location, each laboratory is strongly urged to establish its own reference for this procedure.

## Performance Characteristics

**Comparison:** A comparison study was performed between this method and another manufacturer with the same methodology. Seventy-six samples, ranging in amylase activity from 34 to 2589 U/L were assayed. The resulting correlation coefficient was 0.999 and the regression equation was Y = 1.01X - 2.1

**Precision:**

**Within Run**

| Mean | S.D. | C.V.% |
|---|---|---|
| 40 | 1.5 | 3.8 |
| 364 | 4.5 | 1.2 |

**Run to Run**

| Mean | S.D. | C.V.% |
|---|---|---|
| 40 | 1.3 | 3.3 |
| 353 | 10.7 | 3.0 |

**Linearity:** To 2,000 U/L, samples exceeding this value should be diluted with an equal amount of saline, the assay repeated and results multiplied by 2.

## References


1. Tietz, N.W., Textbook of Clinical Chemistry, W.B. Saunders Co., Philadelphia (1986), 1270.
2. Blair, H.E., U.S. Patent Pending
3. Demetriou, J. et al, Clinical Chemistry: Priniples and Technics, 2nd ed., (Henry, R.J. et al eds.), Hagerstown (MD), Harper & Row (1974).
4. Young, D.S., et al., Clin. Chem. 21:1D (1975).
5. Burtis, C.A., Ashwood E.R., Tietz Textbook of Clinical Chemistry, 2nd Ed., W.B. Saunders Co., Philadelphia, PA (1994) p 2178.



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