For life science research only. Not for use in diagnostic procedures. FOR *IN VITRO* USE ONLY.

# α-Glucosidase Assay

Colorimetric assay for the quantitative determination of α-glucosidase (maltase) in human semen research samples

**Cat. No. 11 742 027 001**

30 assays + 10 blanks

**Version January 2005**

Store at +2 to +8°C

## 1. What this Product Does

**Number of Tests**

30 tests and 10 blanks

**Kit Contents**

| Vial | Label | Contents | |
|---|---|---|---|
| 1 | **Reaction Buffer** Phosphate buffer, pH 6.8 containing 1% SDS | 4 ml | glass bottle blue screw cap |
| 2 | **Substrate Concentrate Solution** containing 4-Nitrophenyl-α-D-gluco-pyranoside in organic solvent | 400 μl | glass bottle red screw cap |
| 3 | **Stopping Buffer** Sodium carbonate buffer | 100 ml | plastic bottle white screw cap |
| 4 | **Inhibitor** Castanospermine, lyophilizate | | plastic vessel green screw cap |
| 5 | **Standard Concentrate Solution** containing 4-Nitro-phenol in organic solvent | 1.5 ml | glass bottle yellow screw cap |

**Storage and Stability**

The kit is stable until expiry date given on the kit label if stored at +2 to +8°C. For stability of reconstituted kit components and working solutions see section 2.1.

**Additional Equipment Required**

The enzyme incubation during the assay is performed at +37°C in a water bath or other incubator.

To measure the α-glucosidase values a photometer with 1.5 ml cuvettes or a microplate reader with a 405 nm wavelenght fliter can be used.

A positive displacement pipette (*e.g.*, Gilson Microman) is necessary for pipetting the highly viscous semen samples.

**Application**

The α-Glucosidase Assay is designed to specifically measure the neutral α-glucosidase in human semen samples **in life science applications. It is not designed for diagnostic applications.** The kit can **not** be used to measure α-glucosidase in other specimens. The assay is compatible with microplate assay format and standard cuvette format.

**Assay Time**

Approx. 2.5 h

## 2. How to Use this Product

### 2.1 Before You Begin

**Preparation and Stability of Solutions**

**Reaction Solution**

For each sample to be analyzed dissolve 10 μl of substrate concentrate solution (bottle 2 warm to +37°C until the content of the bottle is entirely dissolved) in 90 μl reaction buffer (bottle 1). In addition for each series of samples another 100 μl reaction solution is necessary for the castanospermine blank. Prewarm the reaction solution to +37°C in a water bath or other incubator before use.

The reaction solution should be always prepared freshly and is stable for several hours.

**Inhibitor Solution**

Dissolve the lyophilized castanospermine (bottle 4) in 150 μl redist. water.

The inhibitor solution is stable at +2 to +8°C for two months, of stored separately at −15 to −25°C until the expiry date given on the kit.

**Standard Curve**

For each series of samples to be analyzed prepare 10 ml of a 100 μM 4-nitrophenol diluted standard solution by mixing 200 μl of standard concentrate solution (bottle 5 warm to +37°C until the content of the bottle is entirely dissolved) with 9.8 ml of stopping buffer (bottle 3). Prepare the standard curve according the following table:

| μl 100 μM 4-nitrophenol (= standard solution) | μl stopping buffer (bottle 3) | end concentration: 4-nitrophenol standard solution [μM] |
|---|---|---|
| 0 | 1000 | 0 |
| 200 | 800 | 20 |
| 400 | 600 | 40 |
| 600 | 400 | 60 |
| 800 | 200 | 80 |
| 1000 | 0 | 100 |

### 2.2 Assay Protocol

| | |
|---|---|
| 1 | Centrifuge semen sample for 10 min at 1000 × *g* and transfer the clear supernatant (seminal plasma) to a new tube. The seminal plasma can be used either directly or stored at −15 to −25°C. Repeated freezing and thawing of the seminal plasma does not influence seminal α-glucosidase activity. |
| 2 | Place 15 µl of the semen sample into a 1.5 ml reaction vessel by using a positive displacement pipette (*e.g.*, Gilson Microman). Owing to the high viscosity of the seminal plasma other pipettes will cause higher variances.<br>**Optional:** Prepare an internal quality control in the same way (previously measured sample from which aliquots have been stored frozen).<br>Semen samples may be set up in duplicates to decrease variations of the pipetting steps. |
| 3 | Only to one sample of each series (*e.g.*, to a second sample of the internal quality control pool) add 8 µl casanospermine (negative control). |
| 4 | Add 100 µl of prewarmed reaction solution to each sample, mix by vortexing and incubate at +37°C for 2 h. |
| 5 | During incubation prepare standard curve. |
| 6 | Stop incubation by addition of 1 ml stopping buffer and mix by vortexing. |
| 7 | Transfer entire sample to a 1.5 ml cuvette or a 250 µl aliquot into a microplate. |
| 8 | Read the absorbance of the sample ($A_{sample}$) and the absorbance of the seminal plasma blank ($A_{blank}$) respectively at 405 nm against 1 ml stopping buffer. Reading should be completed within 1 h after stopping the reaction. |

### 2.3 Analysis

**Calculation**

Calculate the net absorbance (ΔA) for every sample:

$\Delta A = A_{sample} - A_{blank}$

Determine the inverted slope of the standard curve ((µM/absorbance unit))

V = total volume = 1.115 ml

v = sample volume = 0.015 ml

$$\text{Glucosidase activity in the sample [mU/ml]} = \frac{\frac{1}{\text{Slope}} \times \Delta A \times V}{120 \text{ min} \times v}$$

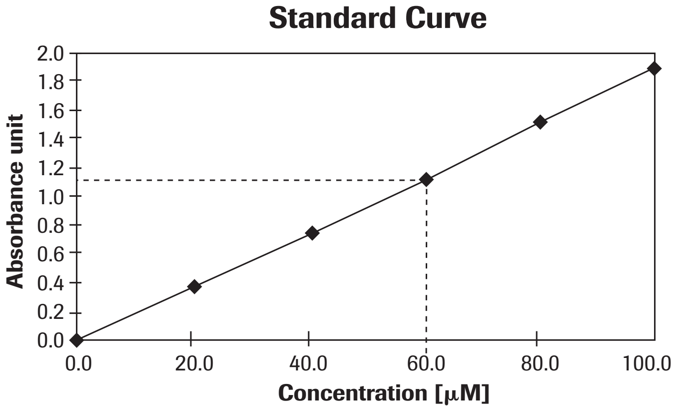
or:

$$\text{Glucosidase activity in the sample [mU/ml]} = \frac{1}{\text{Slope}} \times \Delta A \times 0{,}619$$

## 3. Results

| | |
|---|---|
| measured sample absorbance ($A_{sample}$) | = 1.45 absorbance units |
| semen blank absorbance ($A_{blank}$) | = 0.05 absorbance units |
| Net absorbance of the sample (ΔA) | = 1.4 absorbance units |
| 1/slope of the standard curve | = 60 µM/1.123 absorbance units<br>= 53.4 µM / absorbance unit |
| Glucosidase activity in the sample | = 53.4 x 1.4 x 0.619 mU/ml<br>= 46.3 mU/ml |

⚠ The standard curve should be linear, and so the slope may be determined at any concentration and can be used to multiply all values by the same factor. If the standard curve were to be non-linear and decreasing at higher concentrations (*e.g.*, as may be for some older types of photometers), then a curve should be drawn through the points and the slope is determined directly at the absorbance value of the sample.




## 4. Additional Information on this Product

### 4.1 How this Product Works

This product is designed for use in research studies to elucidate the role of α-glucosidase in male infertility. Literature indicates that the measurement of α-glucosidase in human seminal plasma may be used in the evaluation of male infertility. The normal ejaculate is a heterogeneous mixture of the secretions from three organs of the male genital tract. About 50 to 65% of the volume is secreted by the seminal vesicles with about 30 to 40% contributed by the prostate. Secretion of the testes, epididymides and vasa deferentia makes up 3 to 5% of the total ejaculate (1). Fructose (2) and citrate (3) levels in seminal fluid indicate the activity of seminal vesicles and prostate respectively, whereas carnitine (4) and α-glucosidase seem to be correlated with the epididymal function (5). α-Glucosidase activity is easy to measure and seems to give more significant results than carnitine determinations in individuals with proven epididymal dysfunction (6). Although seminal α-glucosidase activity originates mainly from the epididymis the determination of total α-glucosidase activity would also measure α-glucosidase from other organs (*e.g.*, prostate) and thus lead to erroneous results. This test has been substantially improved by taking advantage of the fact that these enzymes have pH-optima in the acidic range and can be totally inhibited by sodium dodecyl sulfate (SDS) (7). A further improvement was achieved by subtracting non-α-glucosidase-regulated degradation of the substrate as a castanospermine inhibited semen blank (8).

Levels of α-glucosidase activity seems to be very low in that cases of azoospermia and asthenozoospermia, where bilateral ductal occlusions are situated between the epididymis and the ejaculatory duct (9, 10). Low levels of α-glucosidase therefore probably reflect a variety of pathological conditions *e.g.*, agenesis of the vas deferens, varicocoele, infections, inflammations or vasectomy (9). Normal α-glucosidase activity in subjects with azoospermia and asthenozoospermia indicates according to the literature other causes, such as an arrest of sperm maturation, obstructions between the rete testis and the epididymis or obstructions of the rete testis itself.

The **α**-Glucosidase Assay is intended as a tool to increase scientific knowledge about the above relationship.

### 4.2 Test Principle

The α-Glucosidase Assay contains the substrate and all buffers for the measurement of α-glucosidase in human semen or seminal plasma samples. It also contains a standard substance to set up a standard curve. In a first step the semen sample usually is centrifuged and the clear supernatant is used for the assay. Uncleared samples may also be used, but may in some cases lead to higher assay variances. Subsequently the assay is started by adding the substrate in buffer to the sample. The buffer maintains a neutral pH and contains sodium dodecyl sulfate (SDS). Therefor only the neutral α-glucosidase from the epididymis is measured. A blank control sample is set up by mixing the specific inhibitor castanospermine to the sample before adding the substrate. During a 2 hour incubation at +37°C the α-glucosidase containing sample will liberate 4-nitrophenol from the substrate 4-nitrophenyl-α-D-glucopyranoside according to the following equation:

$$\text{4-Nitrophenyl-}\alpha\text{-D-glucopyranoside} \xrightarrow{\alpha\text{-glucosidase}} \text{4-nitrophenol} + \alpha\text{-D-glucopyranoside}$$

4-Nitrophenol yields a yellow colour upon addition of stopping buffer, which is measured in photometer. After subtraction of the castanospermine inhibited semen blank, the enzyme activity is calculeted by comparision to a 4-nitrophenol standard curve.

### 4.3 Assay Characteristics

**Sensitivity**

The sensitivity of the assay is 2 mU/ml. Only 15 µl semen or seminal plasma is necessary due to the high sensitivity.

**Measuring Range**

The assay is linear between 2 mU/ml and 45 mU/ml.

**Precision**

Intra-assay variances are only 1–3%, inter-assay variances are up to 5%.

### 4.4 References


1 Wetterauer, U. (1986) *Urol. Res.* **14**, 241.
2 Schill, W.-B. (1976) *Med. Klin.* **71**, 1031.
3 Hensel, R. & Hornstein, O. P. (1970) *Andrologie* **2**, 61.
4 Wetterauer, U. & Heite, H.-J. (1976) *Akt. Dermatol.* **3**, 93.
5 Paquin, R. et al. (1984) *J. Androl.* **5**, 277.
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7 Tremblay, R. R. (1986) In: Andrology, Male Fertility and Sterility (Paulson, J. D. et al. eds.) Academic Press, London, p. 273.
8 Cooper, T. G. et al. (1990) *Int. J. Androl.* **13**, 297.
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## 5. Supplementary Information

### 5.1 Conventions

**Text Conventions**

To make information consistent and memorable, the following text conventions are used in this package insert:

| Text Convention | Use |
|---|---|
| Numbered Instructions labeled ❶ ❷,etc. | Steps in a procedure that must be performed in the order listed |
| Asterisk * | Denotes a product available from Roche Applied Science |

**Symbols**

In this package insert the following symbol is used to highlight important information:

| Symbol | Description |
|---|---|
| ⚠ | Important Note:<br>Information critical to the success of the procedure or use of the product. |

### 5.2 Changes to Previous Version

Version January 2005 is completely revised.

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