For life science research only.
Not for use in diagnostic procedures.

α-Glucosidase Assay

Version: 08
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Colorimetric assay for the quantitative determination of α-glucosidase (maltase) in human semen research samples.

Cat. No. 11 742 027 001 1 kit
30 assays + 10 blanks
Not available in US

Store the kit at +2 to +8°C.

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1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	blue	α-Glucosidase Assay, Reaction buffer	Phosphate buffer, pH 6.8 containing 1% SDS.	1 glass bottle, 4 ml
2	red	α-Glucosidase Assay, Substrate concentrate solution	Contains 4-nitrophenyl-α-D-glucopyranoside in organic solvent.	1 glass bottle, 400 µl
3	white	α-Glucosidase Assay, Stopping buffer	Sodium carbonate buffer	1 plastic bottle, 100 ml
4	green	α-Glucosidase Assay, Inhibitor	Castanospermine, lyophilized	1 plastic vessel
5	yellow	α-Glucosidase Assay, Standard concentrate solution	Contains 4-nitrophenol in organic solvent.	1 glass bottle, 1.5 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the kit is stable through the expiry date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	blue	Reaction buffer	Store at +2 to +8°C.
2	red	Substrate concentrate solution	
3	white	Stopping buffer	
4	green	Inhibitor	
5	yellow	Standard concentrate solution	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Water bath or other incubator (+37°C)
- Photometer with 1.5 ml cuvettes or microplate reader with a 405 nm wavelength filter
- Positive-displacement pipette, such as Gilson Microman

1.4. Application

The α-Glucosidase Assay measures:
- Neutral α-glucosidase in human semen samples in life science applications.
- The assay is compatible with the microplate assay format and standard cuvette format.

⚠ ***The kit cannot be used for diagnostic applications or to measure α-glucosidase in other specimens.***

1.5. Preparation Time

Assay Time

Approximately 2.5 hours.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Measuring range

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

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Solution	Preparation	Storage and Stability
Reaction solution	▪ For each sample, dissolve 10 µl of Substrate concentrate solution (Bottle 2) in 90 µl Reaction buffer (Bottle 1). ⚠ ***Prewarm Substrate concentrate solution (Bottle 2) to +37°C until fully dissolved prior to using.*** ▪ 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.*	⚠ ***Always prepare fresh; the solution is only stable for several hours.***
Inhibitor solution	Dissolve the lyophilized Inhibitor (Castanospermine, Bottle 4) in 150 µl double-distilled water.	Store 2 months at +2 to +8°C or −15 to −25°C through the expiry date printed on the label.
4-nitrophenol diluted standard solution	For each series of samples to be analyzed in the standard curve, prepare 10 ml of a 100 µM 4-nitrophenol diluted standard solution: ▪ Mix 200 µl of Standard concentrate solution (Bottle 5) with 9.8 ml of Stopping buffer (Bottle 3). ⚠ ***Prewarm Standard concentrate solution (Bottle 5) to +37°C until fully dissolved prior to using.*** ⓘ *See section,* **Protocols, Assay protocol** *for preparation of the standard curve.*	–

2.2. Protocols

Assay protocol

1. Centrifuge semen sample for 10 minutes at 1,000 × *g*.
   – Transfer the clear supernatant (seminal plasma) to a new tube.
   i *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, such as Gilson Microman.
   i *Due to the high viscosity of the seminal plasma, other pipettes may cause higher variances.*
   – Optional: Use aliquots of previously measured samples from which aliquots have been stored frozen to prepare an internal quality control.
   – Semen samples may be set up in duplicates to decrease variations of the pipetting steps.

3. For a negative control, add 8 µl Castanospermine to one sample of each series, for example, to a second sample of the internal quality control pool.

4. Add 100 µl of prewarmed Reaction solution to each sample.
   – Mix by vortexing.
   – Incubate at +37°C for 2 hours.

5. During the 2 hour incubation, prepare the standard curve according to the following table.

100 µM 4-nitrophenol (= standard solution) [µl]	Stopping buffer (Bottle 3) [µl]	Final conc. 4-nitrophenol standard solution [µM]
0	1,000	0
200	800	20
400	600	40
600	400	60
800	200	80
1,000	0	100

6. Stop incubation by adding 1 ml Stopping buffer.
   – Mix by vortexing.

7. Transfer 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 hour after stopping the reaction.

2.3. Parameters

Precision

- Intra-assay variances are only 1 to 3%.
- Inter-assay variances are up to 5%.

Sensitivity

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

3. Results

Calculation

1. Calculate the net absorbance (ΔA) for every sample (Fig. 1).
   $\Delta A = A_{sample} - A_{blank}$

2. Determine the inverted slope of the standard curve (µM/absorbance unit) as shown in Figure 2.
   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:

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

Fig. 1: Glucosidase activity calculation.

Results

Measurement	Absorbance units
Measured sample absorbance (A_{sample})	1.45
Semen blank absorbance (A_{blank})	0.05
Net absorbance of the sample (ΔA)	1.4
1/slope of the standard curve	60 µM/1.123 = 53.4 µM/absorbance unit
Glucosidase activity in the sample	53.4 × 1.4 × 0.619 mU/ml = 46.3 mU/ml

⚠ ***The standard curve should be linear, therefore, the slope may be determined at any concentration and can be used to multiply all values by the same factor. If the standard curve was nonlinear and decreasing at higher concentrations, for example, with older photometers, draw a curve through the points; the slope is determined directly at the absorbance value of the sample.***

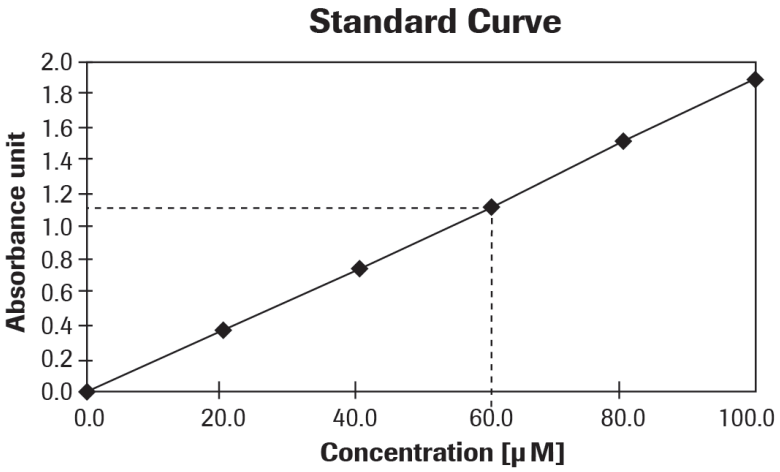

Fig. 2: Standard curve

4. Additional Information on this Product

4.1. 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.

1. Semen sample is centrifuged and the clear supernatant is used for the assay.
   – Uncleared samples may also be used, but may lead to higher assay variances.

2. 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). Therefore, only the neutral α-glucosidase from the epididymis is measured.

3. A blank control sample is set up by mixing the specific inhibitor castanospermine with the sample before adding the substrate.

4. 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 equation in Figure 3.

5. 4-nitrophenol yields a yellow color upon addition of Stopping buffer, which is measured in a photometer.

6. After subtraction of the castanospermine inhibited semen blank, the enzyme activity is calculated by comparison to a 4-nitrophenol standard curve.

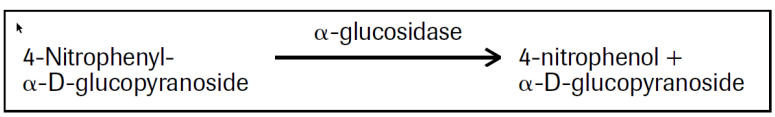

Fig. 3: α-Glucosidase test principle.

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.
- Fructose and citrate levels in seminal fluid indicate the activity of seminal vesicles and prostate respectively, whereas carnitine and α-glucosidase seem to be correlated with the epididymal function.
- α-glucosidase activity is easy to measure and provides more significant results than carnitine determinations in individuals with proven epididymal dysfunction. 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 such as the 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 SDS.
- A further improvement was achieved by subtracting non-α-glucosidase-regulated degradation of the substrate as a castanospermine inhibited semen blank.
- Levels of α-glucosidase activity seems to be very low in cases of azoospermia and asthenozoospermia, where bilateral ductal occlusions are situated between the epididymis and the ejaculatory duct. Low levels of α-glucosidase therefore probably reflect a variety of pathological conditions, such as agenesis of the vas deferens, varicocoele, infections, inflammations, or vasectomy. Normal α-glucosidase activity in subjects with azoospermia and asthenozoospermia indicates 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.

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

5.3. Trademarks

All product names and trademarks are the property of their respective owners.

5.4. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

5.5. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

5.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.7. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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