Sperm 360°

Sperm 360° ™

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α-Glycosidase

bio-marker of epididymis activity

REF SP/SFT/α-011

IVD

User Manual

a

Sperm 360°

product

Turnaround time for test: 140 min

Store at: 2°C - 8°C after receiving

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α - Glycosidase

Bio-marker of epididymis activity

CONCEPT

Poor quality semen may result from testicular production of abnormal sperm or from post testicular damage of sperm in the epididymis or the abnormal ejaculate from accessory gland. Male accessory sex organs made of epithelial / mesenchymal components, require 'Androgen' for proliferation & maintenance of their functions.

The epididymis makes a significant contribution to the development of a fertile sperm, through four classical functions, sperm maturation, sperm transport, sperm concentration & sperm storage.

α-Glycosidase represents as Bio-marker of Epididymis as its major contribution is from Epididymis. The evaluation of α-Glycosidase is regarded as a reliable parameter of Epididymal potency.

Post-testicular masturbation of sperm taking place in the epididymis is now well recorded, makes a significant contribution to the development of fertile ejaculate.

Differential diagnosis of Azoospermia only the determination of the α-Glycosidase is useful.

The reduction of α-Glycosidase in infertile male is to be attributed to reduced sperm count.

1 Specimen Preparation

- Semen sample is collected with :
  - **Abstinence period** of **2-7days**.
  - **Ideal collection** through **masturbation** in sterile container.
  - **Non-spermicidal polyurethane semen collection pouch (Sperm Collect™)** can be used when required.
- Semen sample is allowed to liquefy and then well mixed for performing test.
- **Sperm–free Seminal Plasma Preparation :**
  - **Note down** semen volume (upto one decimal).
  - **Centrifugation** (with Androspin™) of semen sample (liquefied, well mixed) at **3000 rpm** for **10 - 20 min**.
  - **Aspirate supernatant** to obtain seminal plasma & **leave** the **pellet**.
  - Can be stored at **-20°C** for **12** months.

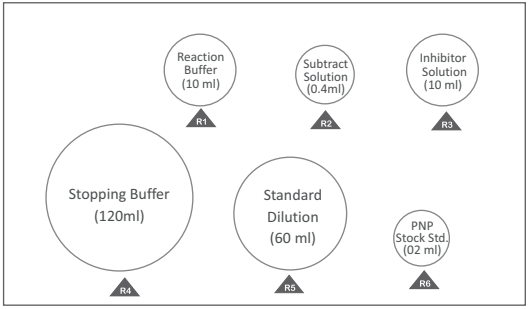
Special Instructions :

- **Hyperviscous** semen sample should be processed to bring towards normal viscosity. (**Viscosity-CH**™ or **Viscosity-BR**™ kit can be used)
- **Frozen semen** plasma must be thawed at 37°C (with Sperm Warmer™) before performing test.

2 Kit Contents

- Reagent 1 (R1) Reaction buffer : 10 mL
- Reagent 2 (R2) Substrate solution (Conc.) : 0.4 mL
- Reagent 3 (R3) Inhibitor solution : 10 mL
- Reagent 4 (R4) Stopping buffer : 120 mL
- Reagent 5 (R5) Standard dilution buffer : 60 mL
- Reagent 6 (R6) PNP Stock Standard (05 mM): 02 mL

Kit Content Layout Diagram :



Storage Conditions :

- The kit should be stored in dark at 2°C - 8°C after receiving.
- Bring all the reagents to room temperature before use.
- Once opened, store reagents in the fridge protected from light.
- Expiry date is printed on the out side of the box.

3 Equipments

REQUIRED BUT NOT PROVIDED IN KIT

- Controlled Temperature 37°C Dry bath (Sperm Warmer™ / Water bath)
- Centrifuge Machine (Androspin™)
- Bio-chemistry Analyser (Androchem Analyser™)
- Pipettes Set
- Stopwatch
- Microtip Box
- Test Tube Stand

4 Disposable Materials

REQUIRED BUT NOT PROVIDED IN KIT

- Hand gloves
- Semen Collection Container
- Non-spermicidal Semen Collection Pouch (Sperm Collect™)
- Microtips
- Pasteur Pipettes
- Test Tubes
- Micro Tubes

5 Bio-chemistry Analyser Settings

Assay Parameters For Programming :

01.	Test Name	: **Alpha Glycosidase**
02.	Mode	: Differential with multi standard
03.	Primary wavelength	: 405 nm
04.	Temperature	: 37° C
05.	Aspiration Volume	: 500 µL
06.	Lag time	: 05 Sec
07.	Blank	: No
08.	Q. C.	: No
09.	Standard	: 05
10.	Concentration	: 0 , 10, 50, 100 & 150 µM
11.	Norm	: Yes
12.	Reagent linearity	: 250 mIU/ mL
13.	Read sec	: --
14.	K factor	: 0.484
15.	Unit	: mIU / mL
16.	Reaction Time	: 120 min
17.	Reagent Blank Abs. max.	: 1.5

AndroChem™ Analyser

(Mfg. by Sperm Processor Pvt. Ltd., Aurangabad, India)

- Is preprogrammed with above mentioned settings
- No additional programming is required.

6 Procedure

Step 1 :
- **Note down semen volume** (upto one decimal).
- **Bring** all the reagents to room temperature before use.

Step 2 : Take **two** micro centrifuge tubes & label them as '**Blank**' **(B)** & '**Test**' **(T)**. (With appropriate **Lab ID No.**)

Step 3 : Take the reagents & put in appropriate tubes with help of air displacement pipettes as per the following table.

Reagents	Test Tube	
	Blank (B)	**Test (T)**
R1 – Reaction buffer	140 µL	-----
R3 – Inhibitor solution	----	140 µL
R2 – Substrate solution	03 µL	03 µL
Seminal Plasma	20 µL	20 µL

- **Incubate tubes at 37°C (Dry Bath) for 2 hrs.**
- **After 2 hrs add following reagent (R4)**

R4 - Stopping Buffer	1000 µL	1000 µL

NOTE :
- Each test preparation require own blank.
- Avoid common blank, whenever there are more than one test.

7 Preparation of Serial Standard Graph

Step A :
- Take a test tube & prepare **Solution - A (Diluted PNP Std.)** with the help of following as per following table.

R6 (PNP Stock Std.)	80µL
R5 (Std. Dilution Buffer)	1920µL
Solution - A (Diluted PNP Std.)	2000µL

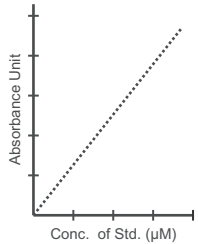
- Mix well & use solution for **Step B**

Step B :
- Take 5 new test tube & label them as **Std.1 (S 1)**, **Std. 2 (S 2)**, **Std. 3 (S 3)**, **Std. 4 (S 4)** & **Std. 5 (S 5)**
- Add reagents to respective test tubes as per table given below & mix well.

Test Tube	**Step A Solution** (Diluted PNP Std.)	Reagent 5 (R5) (Std. dilution buffer)
Std. 1 (0 µM)	-----	1000 µL
Std. 2 (10 µM)	050 µL	0950 µL
Std. 3 (50 µM)	250 µL	0750 µL
Std. 4 (100 µM)	500 µL	0500 µL
Std. 5 (150 µM)	750 µL	0250 µL

Step C :
- First read absorbance of **Std. 1 (S 1)** by using **distilled water** as **blank** .
- Read serially absorbance of **Std. 2 (S 2)**, **Std. 3 (S 3)**, **Std. 4 (S 4)** & **Std. 5 (S 5).**

Step D : Plot **Serial Standard (Std) Graph** as shown below by plotting concentration of Std. on X axis & absorbance on Y axis.



- If **Androchem Analyser** is used, results are displayed automatically.
  If required take print out.

NOTE : Save this Std. Graph & use for test results.

8 Examination

- Read the absorbance by using Bio-chemistry Analyser (at 405 nm), within 60 minutes.
- Read the absorbance of Blank.
- Prepare the serial standard graph.
- Read the absorbance of test.

NOTE : For each test prepare new blank & avoid using common blank.

Calculations

α-Glycosidase in semen per ejaculate

1. α-Glycosidase in semen (mIU/mL)

A. If Biochemistry Analyser is used :

- α-Glycosidase in semen (**X**) mIU / mL :
  [Abs. T – Abs.B] x (Std. curve factor) x 0.484
  = X mIU / mL

Note : Correction factor 0.484 is calculated on the basis of sample dilution & incubation time.

- Final Result
  α-Glycosidase in semen sample = **X** mIU/mL

B. If *AndroChem Analyser* is used :

AndroChem™ Analyser

(Mfg. by Sperm Processor Pvt. Ltd., Aurangabad, India)

- Is preprogrammed with above mentioned settings.
- No additional programming is required.
- Results are displayed as **X** mIU / mL

9 Result

Quantitative Estimation of α-Glycosidase in Seminal Plasma

(Biomarker - Epididymis)

Volume : ____ mL

Result : ____ mU / mL

: ____ mU / ejaculate

Normal Reference Range :

≥20 mU / ejaculate

(As per fifth edition of WHO laboratory manual for examination and processing of human semen).

Limitations :

- This test provides presumptive quantitative information of α-Glycosidase in seminal plasma
- This parameter should be analyzed by a specialist.
- The result should be evaluated taking into account all clinical & laboratory findings related to the same sample.

10 Precautions

- All patient samples & reagents should be treated as potentially infectious & the user must wear protective gloves, eye protection & laboratory coats when performing the test.
- The kit should be discarded in a proper biohazard container after testing.
- Do not eat, drink or smoke in the area where specimens & kit reagents are handled.
- Do not use beyond the expiration date which appears on the package label.
- It is recommended to use of gloves & face mask.

11 Safety & Environment

- Do not release the products used into the environment. Follow centre guidelines for the storage & disposable of toxic substances.
- Biological samples must be handled as potentially infectious.

Description of Symbols

	consult instructions of use
REF	product reference
LOT	lot number
	use by
	manufacturer
IVD	health surveillance device for in-vitro diagnostic
	contains sufficient for 'n' tests
	temperature limitation
	keep dry
CE	CE mark (Conformité Européene)

Accreditations & Registered Certificates

- **ISO 13485 : 2003** Certified
- CE Certified
- **GMDN** Registered
- **US FDA** Registered

For more information & procedure videos

www.spermprocessor.com/sft-alpha-glycosidase.html

www.youtube.com/watch?v=2dfXlVBjC-4