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EpiScreen Plus™

Diagnostic kit to determine neutral alpha-glucosidase activity in human semen and seminal plasma

IVD

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MATERIAL INCLUDED

Catalogue number

EPI_PLUS EpiScreen Plus - 25 tests

CUSTOMER-TECHNICAL SUPPORT

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CE 2797

EpiScreen Plus™

For in vitro diagnostic use only.
Reagent for professional use only.

GENERAL INFORMATION

EpiScreen Plus™ may assist in the diagnosis and management of male infertility. This assay can be used to determine the neutral alpha-glucosidase activity in semen (plasma), an enzyme which is mainly secreted by the epididymis[1].
The activity of this enzyme is a reliable marker for epididymis function in patients with (very) low sperm concentration or azoospermic patients, having a normal androgen blood level:

- very low activity indicates a bilateral obstruction between the epididymis and the ejaculatory duct[2].
- low activity may reflect partial obstruction of the epididymis [2].
- normal enzyme activity is expected when there is an obstruction above the area in which the enzyme is secreted or in cases of non-obstructive azoospermia (testicular disfunction)[2,3].

INTENDED PURPOSE

EpiScreen Plus™ is a semi-quantitative, non-automated, photometric and diagnostic kit for detecting neutral alpha-glucosidase in human semen or seminal plasma and may be useful for the diagnosis and the management of male infertility.
One EpiScreen Plus™ kit is designed for 25 tests.

TEST PRINCIPLE

The principle of the test is based on the following reaction:

Under specified conditions (pH=6.8; T=37°C), 1 IU of alpha-glucosidase liberates 1 µmol PNP per minute from substrate PNPG[5]. The yellow colour of PNP can be measured spectrophotometrically at 405 nm. Alpha-glucosidase activity is expressed as IU/l (or mIU/ml).

Note: *The reaction buffer contains SDS, which selectively inhibits the acid form of alpha-glucosidase originating from the prostate. This allows specific determination of neutral enzyme activity[4].*

Note: *Because background variance of semen samples is quite large (+/- 20%), we recommend to prepare a negative control for each semen (plasma) sample using the inhibition solution. This inhibitor solution contains glucose, which inhibits the alpha-glucosidase activity[6].*

MATERIAL INCLUDED IN THE KIT

- Reagent 1 (5ml): reaction buffer (pH 6.8), supplemented with 1% SDS
- Reagent 2 (0.25ml): 50x substrate solution (PNPG in DMSO)
- Reagent 3 (5ml): inhibitor solution (reaction buffer containing glucose)
- Reagent 4 (60ml): stopping buffer (0.02M NaOH)
- Reagent 5 (1ml): standard stock solution (5mM PNP)
- Reagent 6 (60ml): standard dilution buffer (0.02M NaOH + 0.1% SDS)

A certificate of analysis and MSDS are available on request or can be downloaded from our website (www.fertipro.com).

MATERIAL REQUIRED, BUT NOT PROVIDED

Plate reader, photometer (405nm filter), thermoshaker, heat block or warm water bath, pipette with fresh tips, 1.5ml Eppendorf tubes, microtiter plate

METHOD

Scan barcode (or follow link on www.fertipro.com) to view the demonstration video.

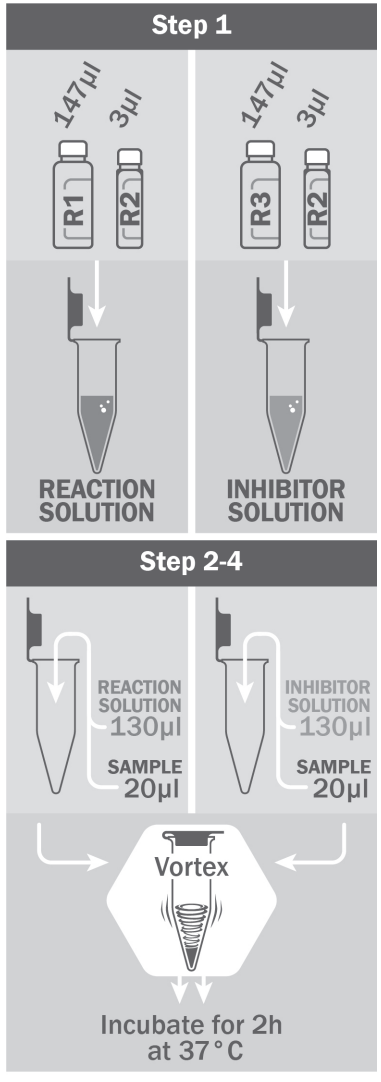
Specimen
Standard semen collection containers should be used, typically in polypropylene and sperm survival/sperm motility tested, when semen is collected by masturbation. Non semen-toxic plastic condoms should be used when semen collection by masturbation is not possible. Centrifuge the semen sample e.g. at 3000g for 10-15 minutes to obtain sperm-free seminal plasma.

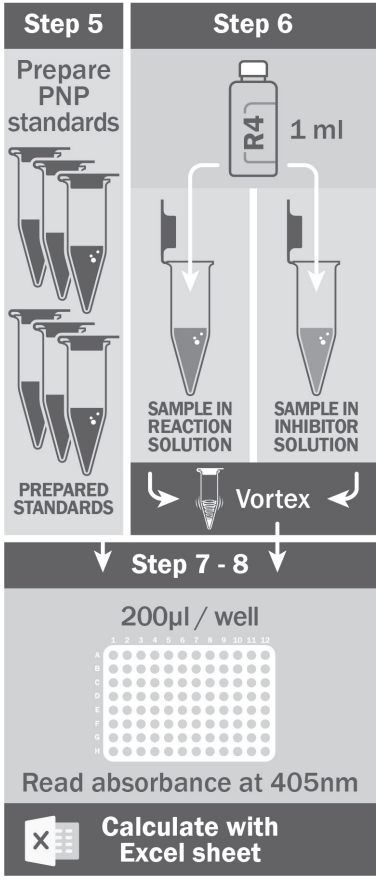
The assay can be performed on fresh or frozen/thawed semen and seminal plasma samples.

Reagent preparation
Do not use the product if seal of the bottles is opened or defect when the kit is delivered.
Warm reagents 1, 2 and 3 up to 37°C for 30 minutes. (**Note:** *precipitation may occur in Reagent 1 but disappears by pre-warming)*

Method Episcreen Plus

Graphic presentation of the protocol and description:





1. For each semen (plasma) sample to be analyzed:
   - *make reaction solution:* 3µl of Reagent 2 (substrate solution) in 147µl of Reagent 1 (reaction buffer)
   - *make inhibitor solution:* 3µl of Reagent 2 (substrate solution) in 147µl of Reagent 3 (inhibitor solution)
2. Pipette 20µl of each semen (plasma) sample into two 1.5ml Eppendorf tubes;
3. Add 130µl reaction solution to one reaction vessel and 130µl inhibitor solution to the other (for negative control);
4. Vortex and incubate for exactly 2h at 37°C in a thermo-regulated warm water bath, a fitting reaction tube thermoshaker or heat block (avoid using an air incubator: this may impair assay outcome!);
5. During incubation of the semen (plasma) samples, prepare the dilutions for the PNP-standard curve:

a. Make the highest standard of 200 µM: dissolve 100 µl of Reagent 5 (standard stock solution) in 2400µl of Reagent 6 (standard dilution buffer). Mix gently.
b. Use this solution to prepare the other standards, as indicated in the table below. Reagent 6 alone serves as 0 µM PNP standard (blank).

Standard dilutions of PNP

PNP standards	200 µM Standard	Reagent 6
200 µM	500 µl	0 µl
150 µM	375 µl	125 µl
100 µM	250 µl	250 µl
50 µM	125 µl	375 µl
10 µM	25 µl	475 µl
0 µM (= blank)	0 µl	500 µl

6 After 2h incubation of the samples (reaction and inhibitor), stop the reaction: remove the tubes from the heat block/warm water bath/ thermoshaker, add 1ml of Reagent 4 (stopping buffer) and vortex;.
7 Pipette 200µl of all samples and standards (prepared in step 5) into a microtiter plate. Preferably, perform this in duplicate.
8 Read absorbance in a photometer at 405nm.
9 After each individual test, all used reagents and materials should be discarded.

CALCULATION/ INTERPRETATION OF RESULTS

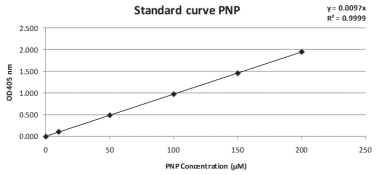
Download the Excel calculation sheet from our website and enter data in the sheet to calculate results:

Principle:

1 Average the duplicate reading for each standard and sample.
2 Subtract the mean absorbance value of the blank (0 µM PNP standard) from all standard readings. These are the blank-corrected absorbances. Only use these blank-corrected values in the next calculations.
3 Calculate the PNP-standard curve (standard concentrations in the X-axis and the blank-corrected OD values in the Y-axis). Perform linear regression to calculate the slope. Coefficient of determination (R2) should be ≥ 0.99.
4 For each reaction sample: subtract the seminal plasma background ($OD_{REACTION}$ – corresponding $OD_{INHIBITOR}$). These are the background-corrected absorbances of your samples.
5 Use equation of the regression curve to calculate PNP concentration of the unknown sample (PNP concentration = background-corrected OD value / slope).
6 Calculate enzyme activity (in mIU/ml) by multiplying the PNP concentration with 0.479 (more information on how the "correction factor" has been determined, can be found in the FAQ on the product page of our website).
7 Normal values for neutral alpha glucosidase in human semen/ seminal plasma: ≥ 5.88mIU/ml.

Example

Assay data and standard curve:



Slope of the curve = 0.0097
(equation curve: y= 0.0097x), R^2 = 0.9999

$OD_{REACTION}$ = 0.845
$OD_{INHIBITOR}$ = 0.060
$OD_{BACKGROUND\ CORRECTED\ SAMPLE}$ = 0.845 – 0.060 = 0.785

Concentration PNP = 0.785 / 0.0097 = 80.93 µM
Enzyme activity per ml = 80.93 µM x 0.479 = 38.76 mIU/ml

LIMITATIONS OF THE METHOD

The EpiScreen Plus is an aid in the diagnosis of male infertility and, as for other biological tests, interpretation of the results must be performed within the framework of clinical findings and data of history taking. Other causes of insufficient epididymal secretion must be excluded, such as hypo-androgenism or severe testicular atrophy.

PERFORMANCE CHARACTERISTICS

Repeatability and reproducibility: CV_{intra} < 15%, CV_{inter} < 15%
Limit of detection: 1.66 mIU/ml
Measuring range: 5.02 -95.8 mIU/ml
Cut-off: ≥ 5.88mIU/ml

STORAGE / DISPOSAL

- EpiScreen Plus is stable for 24 months from the date of manufacture (even after opening).
- Do not use the product after expiry date.
- Store reagents between 2 °C and 8 °C.
- Do not freeze.
- Keep away from (sun)light.
- Suitable for transport or short term exposure at elevated temperatures (up to 5 days at 37 °C).
- The reagents need to be disposed in accordance with the local regulations for disposal of medical devices.

WARNINGS AND PRECAUTIONS

All human, organic material should be considered potentially infectious. Handle all specimens as if capable of transmitting HIV or hepatitis. Always wear protective clothing when handling specimens and reagents (gloves, lab coat, eye/face protection). Reagent 1,3 and 5 do contain sodium azide.
Any serious incident (as defined in the "European In Vitro Diagnostic Medical Device Regulation 2017/746") that has occurred should be reported to FertiPro NV and, if applicable, to the competent authority of the EU Member State in which the user and/or patient is established.

BIBLIOGRAPHY

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4 Paquin R, Chapdelaine P, Dubé JY, Tremblay RR (1984) Similar biochemical properties of human seminal plasma and epididymal alpha-1,4-glucosidase. J. Androl., 5: 227-282
5 WHO laboratory manual for the examination and processing of human semen, sixth edition. Geneva: World Health Organization; 2021
6 Yao X, Mauldin R, Byers L. (2003) Multiple sugar binding sites in α-glucosidase. Biochim. Biophys. Acta, 1645: 22-29

SYMBOLS GLOSSARY

Symbols as defined in ISO 15223	
REF	Catalogue number
LOT	Batch code
	Consult instructions for use
	Manufacturer
IVD	In Vitro Diagnostics
°C	Temperature limit
	Use-by date
	Keep away from sunlight
Symbol as defined in IVDR 2017/746	
CE 2797	CE marking by Notified Body 2797

Other languages can be downloaded on our website (www.fertipro.com)