EpiScreen Plus™

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

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. The activity of this enzyme is a reliable marker for epididymis function in patients with (very) low sperm concentration or azospermic patients, having a normal androgen blood level. 
- very low activity indicates a bilateral obstruction between the epididymis and the ejaculatory duct;
- low activity may reflect partial obstruction of the epididymis;
- 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 dysfunction)

INTENDED PURPOSE

EpiScreen Plus™ is a semi-quantitative, non-automated, photometric, and diagnostic kit for the determination of neutral enzyme activity.

TEST PRINCIPLE

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

\[ \text{PNP} + \text{α-D-glucopyranoside} \rightarrow \text{PNP} + \text{α-D-glucopyranoside} \]

Under specified conditions (pH=6.8; T=37°C), 1 µM of α-glucosidase liberates 1 µM of PNP per minute from substrate PNPG. The yellow colour of PNP can be measured spectrophotometrically at 405 nm.

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.

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.

MATERIAL INCLUDED IN THE KIT

- Reagent 1 (5ml): reaction buffer (pH 6.8), supplemented with 1% SDS
- Reagent 2 (0.25ml): 50x substrate solution (PNP 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), thermostated incubator 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. Do not use the product if seal of the bottles is opened or defect when the kit is delivered.

Prepare reaction solution and inhibition solution:

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 thermostated incubator.

5. During incubation of the semen (plasma) samples, prepare the dilutions for the PNP-standard curve.

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Step 1

Prepare PNP standards

Step 2

Prepare reaction solution

Step 3

Prepare inhibition solution

Step 4

Incubate for 2h at 37°C

Step 5

Read absorbance at 405nm

Step 6

Calculate with Excel sheet

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Note:

- Vortex and incubate for exactly 2h at 37°C in a thermostated incubator.
- A fitting reaction tube thermostaker or heat block (avoid using an air incubator: this may impair assay outcome).
- During incubation of the semen (plasma) samples, prepare the dilutions for the PNP-standard curve.

Note:

- Vortex and incubate for exactly 2h at 37°C in a thermostated incubator.
- A fitting reaction tube thermostaker or heat block (avoid using an air incubator: this may impair assay outcome).
- 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 6 (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

<table>
<thead>
<tr>
<th>PNP standards</th>
<th>200 µM Standard</th>
<th>Reagent 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 µM</td>
<td>500 µl</td>
<td>0 µl</td>
</tr>
<tr>
<td>150 µM</td>
<td>375 µl</td>
<td>125 µl</td>
</tr>
<tr>
<td>100 µM</td>
<td>250 µl</td>
<td>250 µl</td>
</tr>
<tr>
<td>50 µM</td>
<td>125 µl</td>
<td>375 µl</td>
</tr>
<tr>
<td>10 µM</td>
<td>25 µl</td>
<td>475 µl</td>
</tr>
<tr>
<td>0 µM (blank)</td>
<td>0 µl</td>
<td>500 µl</td>
</tr>
</tbody>
</table>

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. Pipette 200µl of all samples and standards, as indicated in the table below. (prepared in step 5) into a microtiter plate. Preferably, perform this in duplicate.

10. Add 1ml of Reagent 4 (stopping buffer) and vortex.

11. Use this solution to prepare the other standards, as indicated in the table below. Reagent 6 alone serves as 0 µM PNP standard (blank).

### EXPERIMENTAL DATA

Example

Assay data and standard curve:

- Slope of the curve = 0.0097
- Concentration PNP = 0.785 µM x 0.479 = 0.3876 mIU/ml

12. Pipette 200µl of the standards, as indicated in the table below.

<table>
<thead>
<tr>
<th>Standard PNP concentration</th>
<th>µM pNP</th>
<th>µl Reagent 5</th>
<th>µl Reagent 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 µM</td>
<td>0 µl</td>
<td>0 µl</td>
<td>0 µl</td>
</tr>
<tr>
<td>150 µM</td>
<td>125 µl</td>
<td>375 µl</td>
<td>0 µl</td>
</tr>
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<td>475 µl</td>
</tr>
<tr>
<td>0 µM (blank)</td>
<td>0 µl</td>
<td>500 µl</td>
<td>0 µl</td>
</tr>
</tbody>
</table>

13. Add 1ml of Reagent 4 (stopping buffer) and vortex.

14. The reagents need to be disposed in accordance with the local regulations for disposal of medical devices.

15. EpiScreen Plus is stable for 24 months from the date of manufacture (even after opening).

16. Do not use the product after expiry date.

17. Store reagents between 2°C and 8°C.

18. Do not freeze.

19. EpiScreen Plus is suitable for transport or short term exposure at elevated temperatures (up to 5 days at 37°C).

20. The reagents need to be disposed in accordance with the local regulations for disposal of medical devices.

### 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 (ODblank) = corresponding OD(Reagent 6) of 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).

### PERFORMANCE CHARACTERISTICS

- Reproducibility: CV<sub>assay</sub> < 15%, CV<sub>within</sub> < 15%
- Limit of detection: 1.66 mIU/ml
- Measuring range: 5.02 - 95.8 mIU/ml
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- Measuring range: 5.02 - 95.8 mIU/ml

### LIMITATIONS OF THE METHOD

- The PNP activity concentration of the unknown samples is expressed in mIU/ml.
- The Linearity of the standard curve was tested. All points were within the limits of the standard curve.
- The correction factor of 0.479 can be derived from the regression curve.
- The assay is not suitable for use with samples that contain hemoglobin, bilirubin or other substances that may interfere with the assay.

### 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.

### BIBLIOGRAPHY