EpiScreen Plus™
NEUTRAL ALPHA-GLUCOSIDASE ASSAY (25 TESTS) - IN VITRO
DIAGNOSTIC DEVICE FOR THE QUANTITATIVE MEASUREMENT OF NEUTRAL
ALPHA-GLUCOSIDASE IN HUMAN SEMEN (PLASMA)

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ABBREVIATIONS

CLSI  Clinical and Laboratory Standards Institute
CV  Coefficient of Variation
IVD  In Vitro Diagnostic Device
LOD  Limit Of Detection
LOQ  Limit Of Quantification
OD  Optical Density
PNP  Para (4)-Nitrophenol
PNPG  Para (4)-Nitrophenyl-alpha-D-glucopyranoside
SDS  Sodium dodecyl sulfate
WHO  World Health Organization

INTENDED USE

EpiScreen Plus™ is an In Vitro Diagnostic Device (IVD) for the quantitative measurement of neutral alpha-glucosidase in human semen (plasma). The enzymatic activity of at least 25 samples can be assessed with one EpiScreen Plus™ kit. Only for professional use.

GENERAL INFORMATION

The bulk of alpha-glucosidase activity in semen, and more particularly that of its neutral iso-enzyme, depends on secretion by the epididymis. In patients with azoospermia and normal androgen levels in peripheral blood, neutral alpha-glucosidase activity in semen plasma is a reliable marker of the epididymal contribution to the ejaculate.

Azoospermic males with bilateral obstruction between the epididymis and the ejaculatory duct will per minute.

Under specified conditions (pH=6.8; T=37°C), the following reaction:

\[
\text{PNPG} + \alpha\text{-glucosidase} \rightarrow \alpha\text{-D-glucopyranoside} + \text{PNP (yellow)}
\]

Under specified conditions (pH=6.8; T=37°C), 1 IU of alpha-glucosidase will per minute, liberate 1 µM of PNP from substrate PNPG. The yellow colour of PNP can be measured spectrophotometrically at 405 nm. Alpha-glucosidase activity is expressed as IU/Liter (or mIU/mL).

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.

Inhibiting Glucose inhibits alpha-glucosidase by binding to the monosaccharide binding site of alpha-glucosidase. This inhibition process is a pH and dose-dependent phenomenon, and is the principle behind creating control semen (plasma) samples.

SPECIMEN TYPES

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

MATERIAL INCLUDED IN THE KIT

The enzymatic activity of at least 25 samples (including sample background correction) can be assessed with one EpiScreen Plus™ 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 PNPG)

- 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 NOT INCLUDED IN THE KIT

Plate reader, photometer (405nm filter), thermostaker or warm water bath, pipettor, 1.5ml Eppendorf tubes, microtitre plate

STORAGE, TRANSPORTATION AND STABILITY

Suitable for transport or short term storage at elevated (up to 5 days at 37°C) and very low (up to 2 days at -18°C) temperatures. EpiScreen Plus™ must be stored at 2-8°C, protected from (sun)light, and remains stable for 24 months. Do not use after expiry date. Opened bottles remain stable for 13 months.

ASSAY PERFORMANCE

Validation parameters have been calculated based on the latest CLSI guidelines.10

| Measuring range: 2.32-144 mIU/ml |
|------------------|------------------|------------------|
| Intra-assay CV: 3.08 % | Sensitivity: 96.0 % |
| Inter-assay CV: 10.52% | Specificity: 93.6 % |
| Within-device Precision: Low pool: 0.96 mIU/ml, High pool: 3.70 mIU/ml |
| Cutoff: 6.35 mIU/ml; 20 mIU ejaculate (if corrected for ejaculate volume) |

* vasectomized/normozoospermic

PRE-USE CHECKS

Do not use the product if seal of the container is opened or defective when the product is delivered. When stored between 2-8°C, precipitation may occur in reagent 1 but disappears by pre-warming to 37°C.

METHOD

Before use, allow reagents 1, 2, and 3 to warm up to 37°C for 30min. We recommend a thermostated water bath or reaction tube thermostaker for optimal heating of the samples. DO NOT incubate in an air incubator as this may impair assay outcome.

Note: The WHO advises to apply only two internal quality control samples for blank correction. Because background variance of semen samples is quite large (+/- 20%), we recommend preparing a negative control for each semen (plasma) sample in order to allow correct and reproducible background correction.

Perform the following steps:

1. For each semen (plasma) sample to be analyzed:
   - make reaction solution: 3µl of substrate solution (Reagent 2) in 147µl of reaction buffer (Reagent 1)
   - make inhibitor solution (= for negative control): 3µl of substrate solution (Reagent 2) in 147µl of inhibitor solution (Reagent 3)

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

4. Vortex and incubate for exactly 2h at 37°C

5. During incubation of the semen (plasma) samples, prepare the standard curve of PNP as follows: Make the highest standard (200 µM) by dissolving 100 µl of standard stock solution (Reagent 5) in 2400µl of standard dilution buffer (Reagent 6). Use this solution to prepare the other standards, as indicated in table 1. Reagent 6 alone serves as 0 standard (blank).

<p>| Table 1: Standard curve of PNP |
|------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>200 µM Standard (µl)</th>
<th>Reagent 8 (µl)</th>
<th>Final concentration (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>375</td>
<td>125</td>
<td>150</td>
</tr>
<tr>
<td>250</td>
<td>250</td>
<td>100</td>
</tr>
<tr>
<td>125</td>
<td>375</td>
<td>50</td>
</tr>
<tr>
<td>25</td>
<td>475</td>
<td>10</td>
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<tr>
<td>0</td>
<td>500</td>
<td>0</td>
</tr>
</tbody>
</table>

6. After 2h incubation of the samples (reaction and inhibitor negative controls), stop the reaction by adding 1ml of the stopping buffer (Reagent 4) and vortex

7. Pipette 200µl of all samples and standards (prepared in step 5) into a microtitre plate

8. Read absorbance at 405nm

INTERPRETATION

STANDARD CURVE

1. Calculate Δ OD values (standard OD value - blank OD value (0-standard))

2. Prepare the standard curve using the readings from table 1.

3. Plot the OD values against the concentration of PNP.

4. The concentration of unknown samples can be determined from the standard curve.

5. Calculate the concentration of alpha-glucosidase in the semen plasma sample.

Example:

Reagent 5 (1ml): standard stock solution (5mM PNPG)

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).

Update:

WHO guidelines have been updated and the new guidelines are now available at www.who.int/iris.

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Certified by:

Dr. John Doe

Certification Authority: Laboratory Standards Institute
2. Plot the Δ OD values (Y-axis) against the standard concentrations (X-axis) and perform a linear regression to calculate the slope. Coefficient of determination (R²) should be ≥ 0.99.

An example is shown below.

**UNKNOWN ENZYME ACTIVITY**

1. Calculate Δ OD values for all reaction samples and inhibitor (negative control) samples: (semen sample OD value - blank OD value (0-standard))
2. Calculate the background-corrected OD value of the semen (plasma) sample: Subtract the Δ OD value from the inhibitor negative control sample from the Δ OD value of the corresponding reaction sample
3. Calculate the corresponding PNP concentration by dividing the background-corrected OD value by the slope of the standard curve
4. Finally, enzyme activity (mIU/ml) is obtained by multiplying the PNP value with 0.479 (see section 'correction factor' below).

**Example**

Assay data:
Blank OD (0-standard) = 0.045;
OD sample = 0.845;
OD sample inhibitor (negative control) = 0.060;
Slope standard curve = 0.0097

1. Δ OD sample = 0.845 – 0.045 = 0.800
2. Δ OD sample inhibitor (negative control) = 0.06 – 0.045 = 0.015
3. Background-corrected OD value of sample = 0.800 – 0.015 = 0.785
4. Concentration PNP = 0.785 / 0.0097 = 80.93 µM
5. Enzyme activity = 80.93 x 0.479 = 38.76 mIU/ml

Calculated enzyme activity can be multiplied with ejaculate volume, to evaluate enzyme activity in the whole ejaculate.

**Note:** The standard curve consists of points between 0-200 µM, as most semen samples will have values within this range. Linearity of the curve has been shown up to 300 µM however. If desired, the operator can alter the curve by starting at 300 µM, corresponding to an enzyme activity of 144 mIU/ml. If unknown samples have higher activity, we advise to dilute and retest to confirm experimental data.

**CORRECTION FACTOR**

This factor is obtained by taking into account the sample dilution factor and incubation time (120 min).

The assay uses 20µl of the semen sample, which is diluted to 1150µl (20µl semen sample + 130µl reaction buffer + 1000 µl stop buffer), yielding a dilution factor of 57.5.

One enzyme unit is defined as the formation of PNP per minute. Therefore, the dilution factor has to be divided by 120 to calculate activity per minute. This results in a dilution factor of 0.479.

**WARNINGS AND PRECAUTIONS**

This test is an aid in the diagnosis 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.

All materials have to be utilized in a safe way according to local/national norms.

**BIBLIOGRAPHY**