

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 epididymis¹. 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.

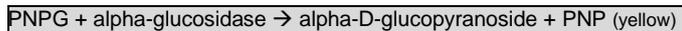
Azoospermic males with bilateral obstruction between the epididymis and the ejaculatory duct have very low alpha-glucosidase activity in their seminal plasma². In contrast, if azoospermia is due to an arrest of sperm maturation, or obstruction situated between the epididymis and the rete testis, or in the rete testis itself, alpha-glucosidase activity is normal. Hence, neutral alpha-glucosidase assessment in seminal plasma of normally virilized men with azoospermia can differentiate between the major causes of this condition^{3,4}.

Low neutral alpha-glucosidase activity in seminal plasma of patients with oligozoospermia may reflect partial obstruction of the epididymides, associated with infections or inflammatory disease^{2,5}. Enzyme activity in patients with normal sperm concentration is correlated with the result of the Shorr-stain of mid-piece and tail, reflecting changes in the sperm membrane, induced by epididymal secretion⁵.

The EpiScreen Plus™ assay may assist in the diagnosis and the management of male infertility.

ASSAY 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 µM PNP per minute from substrate PNPG⁷. 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 activity⁶.

Inhibition: Glucose inhibits alpha-glucosidase by binding to the monosaccharide binding site of alpha-glucosidase⁸. 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 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 NOT INCLUDED IN THE KIT

Plate reader, photometer (405nm filter), thermoshaker 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 CLSI guidelines^{9,10}.

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 defect 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

We recommend to watch our demonstration video (download via link on our website, or scan barcode e.g. with the App "REA PharmaScan"):



Note 1: 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 to allow correct and reproducible background correction.

Note 2: when reagents or samples need to be warmed or incubated, always use a thermo-regulated warm water bath or a fitting reaction tube thermoshaker or heatblock. DO NOT incubate in an air incubator as this may impair assay outcome.

Perform the following steps:

1. Warm reagents 1, 2 and 3 up to 37°C (30 minutes warm water bath).
2. 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**: 3µl of substrate solution (Reagent 2) in 147µl of inhibitor solution (Reagent 3).
3. Pipette 20µl of each semen (plasma) sample into two 1.5ml Eppendorf tubes.
4. Add 130µl reaction solution to one reaction vessel and 130µl inhibitor solution to the other (for negative control).
5. Vortex and incubate for exactly 2h at 37°C in a warm water bath or heatblock.
6. 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 standard stock solution (Reagent 5) in 2400µl of standard dilution buffer (Reagent 6). Mix gently.
 - b. Use this solution to prepare the other standards, as indicated in the table below. Reagent 6 alone serves as 0 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

- After 2h incubation of the samples (reaction and inhibitor), stop the reaction by adding 1ml of the stopping buffer (Reagent 4) and vortex.
- Pipette 200µl of all samples and standards (prepared in step 5) into a microtitre plate.
- Read absorbance at 405nm

CALCULATION OF RESULTS

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

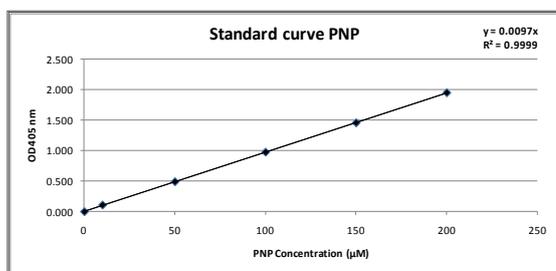
<http://www.fertipro.com/index.php?page=diagnostics&sub=epiplus>

PRINCIPLE:

- Correct all measured OD values for the blank OD value (0 µM PNP standard). Only these corrected values will be further used in the next calculations.
- Calculation of PNP-standard curve, with the standard concentrations in the X-axis and the corrected OD values in the Y-axis. Then, linear regression is performed to calculate the slope. Coefficient of determination (R^2) should be ≥ 0.99 .
- For each reaction sample: correct for its seminal plasma background (= Corrected OD_{REACTION} - Corrected corresponding $OD_{\text{INHIBITOR}}$)
- Use equation of the regression curve to calculate PNP concentration of the unknown sample
(PNP concentration = background-corrected OD value / slope)
- Calculate enzyme activity (in mIU/ml) by multiplying the PNP concentration with 0.479 (see section "correction factor" below)
Calculated enzyme activity can be multiplied with ejaculate volume, to evaluate enzyme activity in the whole ejaculate.

Example

Assay data and standard curve:



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

Blank OD (0 µM PNP standard) = 0.045;

$OD_{\text{REACTION}} = 0.845 \rightarrow$ corrected for the blank: $0.845 - 0.045 = 0.800$

$OD_{\text{INHIBITOR}} = 0.060 \rightarrow$ corrected for the blank: $0.060 - 0.045 = 0.015$

$OD_{\text{BACKGROUND CORRECTED SAMPLE}} = 0.800 - 0.015 = 0.785$

Concentration PNP = $0.785 / 0.0097 = 80.93 \mu\text{M}$

Enzyme activity per ml = $80.93 \mu\text{M} \times 0.479 = 38.76 \text{ mIU/ml}$

Enzyme activity per ejaculate = $38.76 \text{ mIU/ml} \times \text{ejaculate volume (ml)}$

Note 1: 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.

Note 2: the correction factor of 0.479 has been established based on the sample dilution factor and incubation time (120 min):

The assay uses 20µl of the semen sample, which is diluted in reagents to 1150µl, yielding a dilution factor of 57.5. One enzyme unit is defined as the formation of PNP per minute. Therefore, the dilution factor is divided by 120 to calculate activity per minute. This results in a final 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 must be handled in a safe way according to local/national norms.

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TECHNICAL SUPPORT



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