**Fructose Test**

**96 Tests**

**PHOTOMETRIC TEST FOR QUANTIFYING FRUCTOSE IN HUMAN SEMINAL PLASMA**

Document reference: FP09 I29 R01 B.10 Update: 15/02/2018

**Fructose standard contains 0.09% Na-Azide**

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

**GENERAL INFORMATION**

Fructose in semen reflects the secretory function of seminal vesicles. The purpose of the Fructose Test is to measure the amount of Fructose in human semen or seminal plasma. The Fructose Test may help in assessing the diagnosis and the management of male infertility.

**TEST PRINCIPLE**

Fructose reacts, in the presence of HCl under heat, with indole and produces a coloured complex which absorbs at a wavelength of 450-452nm.

**MATERIAL INCLUDED IN THE KIT**

- Reagent 1 - 50ml TCA solution
- Reagent 2 - 25ml Concentrated HCl (32%)
- Reagent 3 - 3ml Indole in methanol
- Reagent 4 - 25ml NaOH (0.5M)
- Fructose Standard - 10ml (5mg/ml)

A certificate of analysis and MSDS are available upon request or can be downloaded from the website.

**MATERIAL NOT INCLUDED IN THE KIT**

Plate reader / photometer (with 450-492nm filter), pipettes and fresh tips, centrifuge tubes, titre plate, centrifuge (> 1000g), small reagent tubes or Eppendorf tubes, water bath or heat block.

**SPECIMEN TYPE**

Perform the test preferably on (frozen/thawed) semen plasma instead of the whole semen sample, especially in cases where the sample is not immediately analyzed (i.e. not within 3 hours after ejaculation). This, to avoid that spermatozoa metabolize fructose leading to an underestimation of fructose concentrations.

**METHOD**

We recommend to view our demonstration video before you first start the test. Hereto, download the video via the link on our website, or scan barcode:

1. Allow the semen sample to liquefy at room temperature
2. Measure total semen/plasma volume (e.g. with a sterile syringe)
3. Pipet 100µL of semen sample/seminal plasma into separate test tubes
4. Pipet 100µL of the Fructose standards (see below how to prepare the standards) into a test tube and treat like a semen sample
5. Add 500µL of Reagent 1 (TCA solution) to the samples and standards and mix
6. Centrifuge for 10 minutes at 1000g or more
7. Blank sample: pipette 20µL of purified water into an empty Eppendorf tube or small reagent tube
8. Add 20µL of Reagent 2 (HCI) to each tube
9. Add 200µL of Reagent 3 (Indole in methanol)
10. Add 500µL of Reagent 4 to the colour reaction
11. Pipet 200µL of sample into an empty well and read results at 450-452nm in a plate reader

**FRUCTOSE STANDARDS**

The kit contains a 5mg/ml Fructose solution. Prepare a standard curve with the following fructose concentrations:

<table>
<thead>
<tr>
<th>Standard</th>
<th>Fructose standard</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>5mg/ml</td>
<td>100µl</td>
<td>0µl</td>
</tr>
<tr>
<td>2.5mg/ml</td>
<td>50µl</td>
<td>50µl</td>
</tr>
<tr>
<td>1mg/ml</td>
<td>20µl</td>
<td>80µl</td>
</tr>
</tbody>
</table>

**Note:** Prepare the dilutions of the standard using purified water (e.g. distilled water).

**INTERPRETATION**

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


The measured value (OD) for the sample is plotted against the standard curve (using the 3 standards mentioned above) with the OD on the Y axes and the concentration of fructose on the X axes. To obtain total Fructose amounts, multiply the result with the total volume of the semen sample or seminal plasma. Normal values according to the WHO manual:

- 2.4mg/ejaculate or more
- 13µmol/ejaculate or more

**ASSAY PERFORMANCE PARAMETERS**

- Intra-assay CV: 8% (Repeatability)
- Inter-assay CV: 13% (Total precision)

**STORAGE**

Suitable for transport or short term storage at elevated temperatures (up to 5 days at 37°C). Store reagents between 2°C and 8°C. Keep away from (sun)light. Product can be used for 12 months after date of production.

The bottle with Reagent 2 (HCl) may show a mild colour change to orange in contact with water. Harmful if swallowed. Avoid contact with skin and eyes. Highly flammable. Toxic by inhalation and if swallowed. Always work under a fume hood when using this reagent.

Reagent 4 (NaOH): Causes burns. Avoid contact with eyes, rinse immediately with plenty of water and seek medical advice. In case of accident or if you are unwell, seek medical advice immediately.

**WARNINGS AND PRECAUTIONS**

Reagent 1 (TCA solution): Causes severe burns. Very toxic to aquatic organisms, may cause long term adverse effects in the aquatic environment. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. In case of accident or if you are unwell, seek medical advice immediately.

Reagent 2 (32% HCl solution): Causes burns. Irritating to respiratory system. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. In case of accident or if you are unwell, seek medical advice immediately. Never add water to concentrated HCl.

Reagent 3 (Indole in methanol): Handling any strong base or acid can cause burns. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. In case of accident or if you are unwell, seek medical advice immediately. Always work under a fume hood when handling strongly concentrated acids or bases. Use protective clothing when handling specimens and reagent (gloves, lab coat, eye/face protection).

**CALCULATION OF G-FORCES**

The g-force of your centrifuge can be calculated using this formula:

\[ g = \frac{1.118 \times r \times \text{rpm}^2}{1000} \]

* rpm = rotations per minute / 1000

\[ r = \text{radius of centrifuge in mm} \]

\[ g = \frac{1.118 \times \text{rpm}^2}{1000} \]

\[ \text{rpm} = \frac{1200}{(1.118 \times 100)} = 3.28 \]

\[ 3280 \text{ revolutions per minute} \]

**BIBLIOGRAPHY**