

FRUCTOSCREEN®

Photometric Method for Detecting Fructose in Seminal Plasma

96 Determinations

Principle:

FructoScreen® measures the amount of fructose in seminal plasma.

In the *first step*, all proteins as well as particulates are separated from the seminal plasma which contains fructose.

In the *second step*, the deproteinized seminal plasma is incubated with indole. The indole changes to a yellow-orange color in the presence of fructose. The intensity of this color can be measured in a microplate reader and is directly related to the amount of fructose in the seminal plasma. Using a formula, both the concentration of fructose and the total amount of fructose present can be calculated.

Reagents:

Solution A: 12 ml zinc solution. Ready to use.

Solution B: 8 ml sodium hydroxide solution. Ready to use. *Warning:* Avoid contact with skin.

Indole: 4 ml indole solution. Ready to use. *Warning:* Avoid contact with skin.

Fructose Standard: 1.5 ml 6.5 mM fructose solution with sodium azide. Ready to use. *Warning:* Dispose of with care.

Empty Dropper Bottle: Capacity for 15 ml concentrated hydrochloric acid with label reading **Concentrated Hydrochloric Acid**. Ready to be filled.

Plate and Lid: 96 wells.

Materials Required But Not Provided:

1. Concentrated hydrochloric acid.
2. Test tubes and rack.
3. Pipettors and tips.
4. Distilled or deionized water.
5. Collecting cups.
6. Centrifuge, capable of 1500g.
7. Air incubator.
8. Microplate reader, capable of reading at 470 nm to 490 nm.

Special Instructions for Filling Empty Dropper Bottle with Concentrated Hydrochloric Acid:

1. Wear gloves and safety goggles.
2. Place your bottle of concentrated hydrochloric acid and the test dropper bottle labeled **Concentrated Hydrochloric Acid** in a venting hood.
3. Remove cap and dropper tip from dropper bottle.
4. Uncap your concentrated hydrochloric acid bottle and carefully remove plastic cork (if present).
5. Pour acid into test dropper bottle. Avoid breathing acid fumes. Avoid spilling acid.
6. Replace plastic cork in your acid bottle and then screw on cap.
7. Holding dropper bottle firmly, force dropper tip into neck of dropper bottle. Screw on cap.
8. In case of contact, flush skin and eyes with copious amounts of water for at least 15 minutes.

Storage and Stability:

Store the **Indole** below 0°C and thaw fully before using. Store the other components at room temperature. They can be used until the expiration date shown on each reagent label. The expiration date is 18 months from the date of manufacture.

Warning and Precautions:

All semen samples should be considered potentially infectious. Handle all specimens as if capable of transmitting HIV and hepatitis. Specimens should be disposed of in accordance with OSHA guidelines.

Avoid inhaling the **Concentrated Hydrochloric Acid** while performing the test. Avoid skin contact with the **Concentrated Hydrochloric Acid**. In the event that the acid gets on your skin or in your eyes, flush with copious amounts of water for at least 15 minutes.

Specimen Collection and Batching:

Semen should be collected in a clean cup. The semen sample can be stored at room temperature until using. Or, semen can be stored frozen by placing specimen in an ordinary freezer until assaying at a later time. No special procedures are needed for freezing and thawing.

Procedure:

1. Allow semen sample to liquefy and measure total volume of semen.
2. Pipette 100 µl semen into a test tube.
3. Add 3 drops of **Solution A** to the test tube.
4. Add 2 drops of **Solution B** to the test tube and mix.
5. Centrifuge 5 minutes at 1500g.
6. Pipette 50 µl supernatant into an empty well of the **Plate**.
7. Pipette 50 µl **Fructose Standard** into an empty well.
8. Pipette 50 µl water, as a negative control, into an empty well.
9. Add exactly 1 drop **Indole** to each well.
10. Add exactly 4 drops **Concentrated Hydrochloric Acid** to each well.
11. Briefly agitate **Plate** (horizontally) to mix contents. Cover **Plate** with **Lid** and incubate 30 minutes at 37°C.
12. Read optical density (OD) of wells at 470 nm (or 490 nm) using a microplate reader. First adjust the microplate reader to zero while reading an empty well.

Calculation of Fructose Concentration:

$$[\text{Fructose}] = \frac{\text{OD}_{\text{seminal plasma}}}{\text{OD}_{\text{Fructose Standard}}} \times 14$$

where 14 is a constant determined by the concentration of fructose in the standard and the dilution of the specimen.

Calculation of Total Amount of Fructose:

$$\text{Fructose} = \frac{[\text{Fructose}]}{1000} \times \text{Volume}$$

Example: At 490 nm the following were obtained for a semen specimen:

Semen Volume = 2 ml
 Mean OD of Specimen = 0.255
 Mean OD of Fructose Standard = 0.168
 OD of Water (negative control) = 0.020

Applying the formula for fructose concentration:

$$\frac{0.255}{0.168} \times 14 = 21.2 \text{ mmol/L [fructose]}$$

Applying the formula for the total amount of fructose:

$$\frac{21.2}{1000} \times 2 = 42.5 \text{ } \mu\text{mol fructose/ejaculate}$$

Performance Characteristics:

Intra-Assay Reproducibility

A semen specimen was assayed 10 times for fructose:

Number	Mean (μmol)	S.D.	C.V.
10	35.8	± 1.60	4%

Selected References:

Sharma N, Acharya A, Singh SK, Singh M, Sharma U, Prasad R. Heterogenous spectrum of CFTR gene mutations in Indian patients with congenital absence of vas deferens. *Hum Reprod* 2009;24:1229-1136.

Casals T, Bassas L, Egozcue S, Ramos MD, Giménez J, Segura A, Garcia F, Carrera M, Larriba S, Sarguella J, Estivill X. Heterogeneity for mutations in the CFTR gene and clinical correlations in patients with congenital absence of the vas deferens. *Hum Reprod* 2000;15:1476-1483.

World Health Organization. *Laboratory manual for the examination of human semen and sperm-cervical mucus interaction*. 4th ed. New York: Cambridge University Press, 1999.

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