

# CITRICSCREEN®

## Photometric Method for Detecting Citric Acid in Seminal Plasma

(96 determinations)

FOR RESEARCH USE ONLY  
NOT FOR USE IN DIAGNOSTIC PROCEDURES

### Principle:

**CitricScreen®** measures the amount of citric acid in seminal plasma.

In the *first step*, semen is treated with alcohol and then centrifuged so as to remove spermatozoa and particulates from the seminal plasma.

In the *second step*, a solution of ferric chloride is added to a known amount of seminal plasma. Any citrate present will cause the solution to turn yellow. The intensity of color can be measured in a microplate reader and is directly related to the amount of citrate ions in the seminal plasma. Using a formula, the total amount of citric acid present can be calculated.

### Reagents:

**Ferric Chloride:** 15 ml ferric chloride solution. Ready to use.

**Alcohol:** 10 ml alcohol. Ready to use.

**Citric Acid Standard:** 1.5 ml 2 mg/ml citric acid solution. Ready to use. *Caution:* Avoid contact with skin and eyes.

**Plate:** 96 wells.

### Materials Required But Not Provided:

1. Distilled or deionized water.
2. Centrifuge, capable of 1500g.
3. Test tubes and rack.
4. Micropipettors and tips.
5. Collecting cups.
6. Microplate reader, capable of reading at 390 - 410 nm.

### Storage and Stability:

Store the reagents at room temperature. They can be used until the date shown on each reagent label.

### Warning and Precautions:

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

### Specimen Collection:

Semen should be collected in a clean cup. The semen sample should 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 liquify and measure total volume of semen.
2. Pipette 100 ul semen sample into a test tube.
3. Add 100 ul **Alcohol** to the test tube and mix.
4. Centrifuge for 10 minutes at 1500g.
5. Pipette 50 ul supernatant into an empty well.
6. Pipette 50 ul **Citric Acid Standard** into an empty well.
7. Pipette 50 ul water, as a negative control, into an empty well.
8. Add exactly 5 drops **Ferric Chloride** to each well.
9. Briefly agitate **Plate** to mix contents.
10. Read optical density (OD) of wells at 390 (or up to 410) nm using a microplate reader. First adjust the microplate reader to zero while reading the negative control and then read the other wells.

*NOTE: The OD reading of the Citric Acid Standard should fall between 0.10 and 0.25. Repeat the test if your Citric Acid Standard is lower (<0.1) or higher (>0.25).*

Calculation of Total Citric Acid:

$$\text{Total Citric Acid} = \frac{\text{OD}_{\text{semenal plasma}}}{\text{OD}_{\text{Citric Acid Standard}}} \times 4 \times \text{Volume}$$

where 4 is a constant determined by the amount of citric acid in the **Citric Acid Standard** and the dilution of the specimen.

Example: At 410 nm the following were obtained for a semen specimen:

Semen Volume = 2.0 ml  
Mean OD of Specimen = 0.23  
Mean OD of Citric Acid Standard = 0.14

Applying the formula:  $\frac{0.23}{0.14} \times 4 \times 2 = 13$  mg citric acid/ejaculate

Performance Characteristics:

Intra-Assay Reproducibility

A semen specimen was assayed 13 times for citric acid:

No.	Mean, mg	S.D.	C.V.
13	6.8	+0.40	6%

## Spiking Recovery

A semen pool was spiked with 0, 1, 2, and 4 mg citric acid and then tested by the **CitricScreen**<sup>®</sup> method. The observed results were determined using a standard curve of citric acid.

	<b>O</b>	<b>E</b>	<b>O/E</b>
<b>Spike, mg</b>	<b>Observed, mg</b>	<b>Expected, mg</b>	<b>Recovery, %</b>
0	0	--	--
1	1.3	1.0	130
2	2.0	2.0	100
4	4.0	4.0	100

Selected Reference:

World Health Organization. 1992. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. Cambridge University Press. Third Edition

BioScreen Inc.

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