

ACROSCREEN™

Photometric Enzyme Method for Detecting Acrosin Activity in Spermatozoa

(96 determinations)

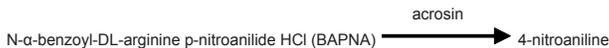
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Principle:

AcroScreen™ measures the total acrosin activity present in spermatozoa relative to the activity of a known amount of protease.

In the first step, a semen sample is subjected to filtration and washing so as to remove the seminal plasma, which contains protease inhibitors, and leave behind spermatozoa.

In the second step, the spermatozoa are incubated with a detergent-substrate solution. The detergent permeabilizes the sperm cell membranes thereby making the intracellular acrosin available to react with the substrate. At the same time proacrosin is converted to acrosin. The acrosin converts the substrate to a soluble colored product:



The intensity of color from semen sample wells is measured in a microplate reader at 405 nm to 410 nm. Using a formula, it is possible to calculate the 'acrosin activity index' for each semen sample by knowing the optical density (OD) of the reacted semen sample, the OD of the positive control, and the number of spermatozoa per volume of semen. Or, using a different formula, it is possible to calculate the acrosin activity per million sperm for each semen sample by knowing the OD of the reacted semen sample and the number of spermatozoa per volume of semen.

Reagents:

10X Wash: 10ml 10X concentrated phosphate buffered saline, pH 7.2, with sodium azide. Dilute 1:10 before using. *Warning:* Dispose of with care.

Substrate: 4 ml N- α -benzoyl-DL-arginine p-nitroanilide hydrochloride (BAPNA) in solvent. Ready to use. *Caution:* Let **Substrate** liquefy *completely* at room temperature before using.

Detergent: 19 ml Triton x-100 in buffer, pH 8.0, with sodium azide. Ready to use. *Warning:* Dispose of with care.

Positive Control: 0.5ml protease in a suspension. Ready to use. Mix thoroughly before using. *Warning:* Contains an eye irritant.

Membrane Plate: Each plate has 96 usable wells. *Caution:* To ensure the usability of all the wells, care should be taken to cover the wells not being used with the **Plate Sealer** so that the membrane does not get destroyed by repeated vacuuming.

Plate Sealer: Peel off backing before using.

Materials Required But Not Provided:

1. Microplate reader, capable of reading at 405 nm to 410 nm.
2. Clear-bottom microplate wells.
3. Test tubes and rack.
4. Pipettors and tips.
5. Vacuum manifold attached to a vacuum source.

6. Microscope or CASA system and counting chamber.
7. Paper towels.
8. Collecting cups.
9. Distilled water, graduated cylinder, 100 ml bottle.

Storage and Stability:

Store the reagents at 2°C to 8°C. 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 Precaution:

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. Semen should be used within three (3) hours of collecting.

Preparation:

1. Bring all reagents to room temperature.
2. Determine the number of wells needed for running the assay. Mark off the wells on the assay map you will be using in the assay. Mark off the wells on the **Membrane Plate**.
3. Cover the wells you will not be using with the **Plate Sealer** after first peeling off backing.
4. Prepare the **Wash:** add 90 ml distilled water to a graduated cylinder and add the 10ml of the **10X Wash** to the water. Mix. This can be stored in a closed bottle and used until the expiration date.

Procedure:

1. Allow semen sample to liquefy.
2. Count spermatozoa.
3. Dilute semen to between 3 million and 6 million spermatozoa per milliliter with **Wash** using the following suggested protocol (note: 100 μ l = 0.1ml):

Sperm Count (million/ml)	Semen Volume	Wash Volume	Dilution	Dilution-Factor*
< 30	100 μ l	+ 400 μ l	1:5	0.1
> 30 - 60	100 μ l	+ 900 μ l	1:10	0.2
> 60 - 120	100 μ l	+ 1900 μ l	1:20	0.4
> 120 - 240	100 μ l	+ 3900 μ l	1:40	0.8
> 240	100 μ l	+ 7900 μ l	1:80	1.6

*Used in formula for calculating the Acrosin Activity Index (AAI).

4. Pipette 100 μ l of the diluted semen into a well according to your assay map. Repeat for duplicates.
5. Pipette 100 μ l of **Wash** into a well as a **Negative Control**.
6. Place **Membrane Plate** onto vacuum manifold and turn on the vacuum gradually. Allow all liquid to drain from wells. Turn off vacuum.
7. Fill up each well with **Wash**. Turn on vacuum. Allow all liquid to drain from wells.
8. Turn off vacuum. Remove **Membrane Plate** and blot its undersurface thoroughly with toweling.
9. Pipette 20 μ l **Positive Control** into a well. Repeat for duplicate.

10. Add exactly 5 drops of **Detergent** to each well.
11. Add exactly 1 drop of **Substrate** to each well.
12. Briefly agitate **Membrane Plate** (horizontally) to mix contents, cover wells and set on a nonabsorbent surface at room temperature. Begin timing.
13. After 90 minutes transfer the entire contents of each well to a clear-bottom microtiter plate well. Be sure there are no bubbles.
14. Read the optical density (OD) at 405 nm to 410 nm within 5 minutes using a microplate reader. First adjust the microplate reader to zero while reading the **Negative Control** and then read the other wells.
15. Record OD readings.

NOTE: The OD reading of the Positive Control should fall between 0.28 and 0.4. Repeat the test if your Positive Control is lower (<0.28) or higher (>0.4). One cause of out of range OD readings can be due to the incubation temperature. The incubation temperature should be room temperature, 20°C to 25°C.

Calculation of Acrosin Activity Index (AAI):

$$AAI = \frac{OD_{\text{diluted semen sample}} \times 10^{10}}{OD_{\text{Positive Control}} \times \text{Sperm Count}} \times \text{Dilution Factor}$$

Example: At 405 nm, the following data were obtained for a semen specimen:

- Sperm Count = 80 X 10⁶ cells/ml
- Dilution Factor = 0.4 for a 1:20 dilution of the semen
- OD of Positive Control = 0.360
- OD of Specimen = 0.146

Applying the formula:

$$\frac{0.146 \times 10^{10} \times 0.4}{0.360 \times 80 \times 10^6} = 20 \text{ (AAI)}$$

Calculation of Acrosin Activity:

Note that this calculation does not use the **Positive Control** data.

$$\mu\text{IU acrosin}/10^6 \text{ sperm} = \frac{OD_{\text{diluted semen sample}} \times \text{Dilution} \times 10^6}{\text{Sperm Count In Millions} \times 278}$$

where Dilution is the amount sample is diluted up; for example, a 1:5 dilution is diluted 5 times, a 1:10 dilution is diluted 10 times, etc. The constant is calculated as follows:

$$278 = \frac{9.9 \text{ mM}^{-1}\text{cm}^{-1} \times 90 \text{ min} \times 0.5 \text{ cm}}{0.18 \text{ ml} \times 10}$$

where 9.9 mM⁻¹cm⁻¹ is the extinction coefficient, 90 min is the incubation time, 0.5 cm is the distance to the cuvette for a microplate reader, 0.18 ml is the volume and 10 is the volume factor of sperm in each well or 1 ml/0.1 ml.

Example: Using the same data from the calculation of Acrosin Activity Index, the following were obtained:

- Sperm Count In Millions = 80
- Dilution = 20 for a 1:20 dilution of the semen
- OD of Specimen = 0.146

Applying the formula using the above example:

$$\frac{0.146 \times 20 \times 10^6}{80 \times 278} = 131 \text{ } \mu\text{IU acrosin}/10^6 \text{ sperm}$$

Selected References:

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