

LEUCOSCREEN™

Cytochemical Stain For Detecting
Granulocytes in Semen

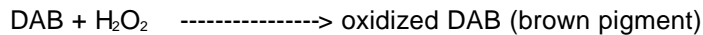
(about 25 - 300 determinations)

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

Principle:

This staining method detects the presence of the enzyme peroxidase in cells.

A semen sample is mixed with a substrate specific for the enzyme peroxidase. If peroxidase is present, it will reduce the substrate, hydrogen peroxide. At the same time, diaminobenzidine (DAB) will be oxidized to form an insoluble brown product:



Using a formula, it is possible to calculate the number of peroxidase-positive cells in each semen sample by knowing the concentration of spermatozoa.

Reagents:

Buffer: 7 ml buffer, pH 7.4. Ready to use.

DAB: 1 ml diaminobenzidine solution. Ready to use. *Warning: Diaminobenzidine is a possible carcinogen.*

Peroxide: 1 ml hydrogen peroxide solution. Ready to use.

Fixative: 12 ml dilute ethanol. Ready to use.

Materials Required But Not Provided:

1. Deionized water.
2. Sperm counting chamber.
3. Glass slides and coverslips.
4. Pipettors and tips.
5. Test tubes and rack.
6. Positive and negative controls (see Controls).
7. Bright-field microscope with 100X - 400X total magnification.

Controls:

For a Positive Control, use any human fluid that contains white blood cells, such as whole blood. Whole blood should be used within 10 minutes of drawing and should contain no additives such as anticoagulants. The tested blood will contain a variety of white blood cells (leucocytes) including some that will be stained brown. Some will be unstained. Thus, the same human fluid specimen preparation will have both a Positive Control, stained brown leucocytes (granulocytes) and a Negative Control, unstained leucocytes, on the same slide.

For a separate Negative Control, run the test but omit the Peroxide. With this method, the tested blood will contain unstained leucocytes.

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.

Storage and Stability:

Store the reagents at 2° - 8°C. They can be used until the expiration date shown on each reagent label.

Specimen Collection:

Semen should be collected in a clean cup. The semen sample can be stored at room temperature until using.

Preparation:

1. Bring all reagents to room temperature.
2. Prepare fresh substrate by adding the following to a test tube:
 - 1 ml water
 - 250 ul **Buffer**
 - 40 ul **DAB**
 - exactly 1 drop **Peroxide**
3. Mix gently. Discard after use.

Procedure:

1. Allow semen sample to liquify.
2. Count spermatozoa.
3. Pipette 20 ul semen into a test tube.
4. Add exactly 1 drop **Fixative**.
5. Pipette 60 ul fresh substrate into the test tube and mix briefly.
6. Prepare specimen for viewing using Method I or Method II.
 - Method I: Pipette 10 - 20 ul onto a glass slide and place a coverslip on top of the liquid. Examine at a total magnification of 400X using a microscope. Count brown cells and sperm within the same viewing area.
 - Method II: Pipette about 5 ul onto a Makler Chamber or Cell-Vu and cover. Examine at a total magnification of 100X using a microscope. Count brown cells within the entire grid area.

Calculation Of Granulocytes In Semen, Method I:

$$\text{Granulocytes/ml} = \text{Sperm Count} \times \frac{\text{Number of brown cells}}{\text{Number of sperm}}$$

Example: The following data were obtained for a tested semen specimen placed on a glass slide with a coverslip and viewed at 400X:

$$\begin{aligned} \text{Sperm Count} &= 73 \times 10^6 \text{ cells/ml} \\ \text{Number of brown cells} &= 4 \end{aligned}$$

Number of sperm = 66
Applying the formula: $73 \times 10^6 \times \frac{4}{66} = \sim 4 \times 10^6$ granulocytes/ml

Calculation Of Granulocytes In Semen, Method II:

Granulocytes/ml = Number of brown cells X 5 X counting chamber factor where 5 is the dilution factor because semen was diluted 20 ul in a total volume of 100 ul.

Example: The following data were obtained for a tested semen specimen viewed at 100X in a Cell-VU:

Number of brown cells in 100 squares = 16
Cell-VU chamber factor = $10^5 / 2$ cells/ml
Applying the formula: $16 \times 5 \times \frac{10^5}{2} = 4 \times 10^6$ granulocytes/ml

Selected Reference:

World Health Organization. 1999. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. Cambridge University Press. Fourth Edition.