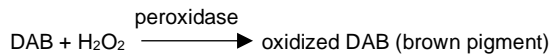


**INTENDED USE**

LeucoScreen™ is a cytochemical staining kit used to detect granulomas in semen samples. The results obtained should be considered as provisional and should not be used in isolation but together with all the information available.

**SUMMARY**

This staining method is used to detect 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.

**PRECAUTIONS**

Diaminobenzidine (DAB) is a possible carcinogen. Use appropriate care in handling this reagent. All semen samples should be considered potentially infectious. Handle all specimens as capable of transmitting HIV or hepatitis. Specimens should be disposed of in accordance with OSHA guidelines. product should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimen, containers and media after use. Directions should be read and followed carefully.

**STABILITY AND STORAGE**

LeucoScreen is stable to the stated expiration date when stored at the required temperature. Store reagents at 2–8°C.

**USER QUALITY CONTROL**

Any product showing cloudiness, turbidity, precipitation, or coloration should be discarded. Run a positive control when preparing fresh substrate.

**SPECIMEN COLLECTION AND PREPARATION**

Semen should be collected in a clean collection cup. The semen sample can be stored at room temperature until the time of use.

**PROCEDURE**

**Materials Provided with LeucoScreen (#0006044):** Contains reagents necessary for 25-300 determinations. There is one bottle or tube of each reagent: Leuco Buffer, DAB (diaminobenzidine solution), Peroxide, Fixative, Peroxidase.

**Materials Not Provided:** Deionized water, sperm counting chamber, glass slides and coverslips, pipettors and tips, test tubes and rack, positive and negative controls (see controls), bright-field microscope with 100X to 400X total magnification.

**Preparation**

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

**Procedure**

1. Allow semen sample to liquefy.
2. Count spermatozoa.
3. Pipette 20 µl semen into test tube.
4. Pipette 20 µl Peroxidase, a positive control into a second test tube.
5. Pipette 20 µl water, a negative control, into a third test tube.
6. Add exactly 1 drop Fixative to each test tube.

7. Pipette 60 µl fresh substrate into each test tube and mix briefly.
8. Observe the test tubes with Peroxidase and water and note any color change. The test tube with Peroxidase should turn dark brown. This indicates that the fresh substrate is working properly. Proceed with the next step if the fresh substrate is working properly.
9. Prepare specimen for viewing using Method I or Method II.
  - Method I:** Pipette 10 µl to 20 µl 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 µl onto a Makler Chamber or a disposable counting chamber. 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:

Sperm Count =  $73 \times 10^6$  cells/ml

Number of brown cells = 4

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 20µl in a total volume of 100 µl.

**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

**EXPECTED RESULTS**

If granulomas are present in the semen cells and processed according to the procedures listed within this document, the granuloma cells should stain brown.

**LIMITATIONS OF PROCEDURES**

Ensure that the reagents are performing properly with the use of positive and negative controls. If the expected result is not present with the controls, the semen sample may show false positive or negative results.

**BIBLIOGRAPHY**

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

**CONTACT**

Alpha-Tec Systems, Inc. offers a complete line of reagents, stains, and QC1™ Quality Control Slides for AFB, Parasitology, Bacteriology, and Mycology processing. Alpha-Tec also provides O&P collection systems and concentration devices for Parasitology, as well as products for the evaluation of male fertility. For Technical Assistance, email [Technical@AlphaTecSystems.com](mailto:Technical@AlphaTecSystems.com), and for Customer Service, email [Sales@AlphaTecSystems.com](mailto:Sales@AlphaTecSystems.com), or call either [+1] 800.221.6058 (USA) or [+1] 360.260.2779 between 8AM and 4PM Monday through Friday, Pacific Time.

**WARRANTY**

This product is warranted by Alpha-Tec Systems, Inc. to perform as described in the labeling and literature supplied. Alpha-Tec Systems, Inc. disclaims any implied warranty or merchantability or fitness for any other purpose, and in no event shall Alpha-Tec Systems, Inc. be liable for any consequential damages arising out of aforesaid express warranty.

**TRADEMARKS:**

LeucoScreen™ is a trademark of Alpha-Tec Systems, Inc., a CalibreScientific Company. 1311 SE Cardinal Court, Suite 170, Vancouver, WA 98683 USA.

**PRODUCT CODES:**

0006044 LeucoScreen, 25-300 determinations



Manufactured by Alpha-Tec Systems, Inc.  
 1311 SE Cardinal Court, Suite 170  
 Vancouver, WA 98683 USA

**GLOSSARY OF SYMBOLS**



Batch code / Numéro de lot / Número de Lote / Numero di lotto / Lot Nummer / Lotnummer / Lotnummer / Šaržna številka / Número de lote



Catalog number / Référence du catalogue / Número de catálogo / Numero di catalogo / Katalognummer / Catalog number / Het aantal van de catalogus / Kataloška številka / Número de catálogo



In vitro diagnostic medical device / Pour usage diagnostique in vitro / Para uso diagnóstico in vitro solamente / Solo per uso diagnostico in vitro / Nur zur Verwendung als in vitro-Diagnostikum / Alleen voor in vitro diagnostisch gebruik / För invitrodiagnostik enbart / Samo za invitro diagnostiko / Apenas para uso em diagnóstico in vitro



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Use-by date / Utiliser avant la date de péremption indiquée / Use antes de la fecha indicada / Utilizzare entro la data indicata / Bis zum angegebenen datum verbrauchen / Gebruik door vermelde datum / Använd innan angivet datum / Porabiti do navadenega datuma / Usar até à data indicada



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Temperature limit / Conserver aux températures indiquées / Almacene entre las temperaturas indicadas / Conservare a temperatura comprese fra quelle indicate / Im angegebenen temperaturbereich aufbewahren / Opslaan bij een temperatuur tussen / Förvara mellan angivna temperaturer / Shranjevati med navedenimi temperaturami / Armazene entre as temperaturas indicadas



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