



SPERM VIABILITY STAIN KIT Catalog #SA200

Fertility Solutions
4635 Richmond Road, Suite 100
Warrensville Hts. OH USA 44128
Telephone 216-491-0030 x200 Fax 216-491-0032

INTENDED USE: For in-vitro diagnostic use to determine sperm viability.

PRODUCT DESCRIPTION: 10 mL 1% Eosin-Y solution, 15 mL 10% Nigrosin solution, dropper caps

WARNINGS AND PRECAUTIONS: For in vitro use only
Users should read the Material Safety Data Sheet, which is available at fertilitysolutions.com/downloads.

STORAGE AND STABILITY: Store at room temperature. Do not use after the labeled expiration date.

MATERIALS NEEDED:

plastic disposable transfer pipets
vial or 2" x 2" square of laboratory plastic film wrap
(e.g. Parafilm®)
frosted 3" x 1" glass slides

22 x 50 mm coverslips
permanent mounting media

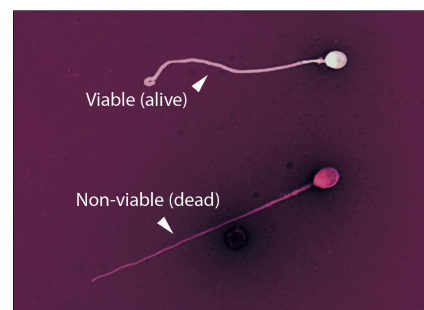
brightfield microscope with 40X objective

MATERIALS RECOMMENDED:

0 -100 µL micropipette with disposable tips

PROCEDURE:

1. Replace the black caps on both bottles with the white dropper caps before the first use.
2. Label 2 frosted end slides following the laboratory procedure for specimen identification.
3. Vortex the specimen then aliquot 1 drop of semen into a vial or onto a plastic square.
4. Mix the Reagent #1: Eosin Y stain by inverting several times. Add 2 drops to the semen. Stir with wooden stirrer for about 15 seconds.
5. Mix the Reagent #2: Nigrosin stain by inverting several times. Immediately add 3 drops to the semen-eosin mixture and stir with the wooden stirrer for about 15 seconds.
6. Transfer an aliquot of the mixture onto the slide. We recommend using 20 µL.
7. Make two smears using your laboratory's procedure. We recommend
 - a. Holding the other labeled slide at a 45° angle to the flat slide, pull the 2nd slide back into the drop and allow the sample to spread evenly and completely along the back edge.
 - b. Use slow, even pressure to push the spreader slide until the sample has been smeared evenly along the entire length of the slide. The sample should never be in front of the 2nd slide edge. It should be pulled behind. If the sperm concentration is less than 20x10⁶/mL, use a greater angle by raising the rear of the smearing slide to obtain a higher distribution of cells. If the concentration is greater than 60x10⁶/mL, use a smaller angle by lowering the rear of the smearing slide. A higher angle and faster smearing will make thicker smears.
8. Allow slides to air dry. Do not expose to condensation or refrigeration because the aqueous eosin will elute onto the smear, making viable sperm look pink.
9. Analyze 200 intact sperm with head and tail using 40x objective. Tally viable sperm whose heads are completely white or very pale pink (note acrosome may not stain). If neck only is pink, tally as viable. Tally non-viable sperm whose heads are stained dark pink or partially (approximately half) dark pink.
10. % Viability = $\frac{\text{Number of Viable Sperm}}{\text{Total Number of Sperm Evaluated}} \times 100$



PROCEDURE QUALITY CONTROL: Use Sperm Viability Control AQC107

REFERENCES:

Rothmann SA and Reese AA. The Andrology Trainer: Fourth Edition. Fertility Solutions Inc., Cleveland, 2010.