

## Eosin-Nigrosin Stain for Sperm Vitality – Technical Memo

**SOLUTIONS:**Eosin Y Stain 0.5%, Aqueous NaCl  
Nigrosin Stain 10%, Aqueous

500 ml

Part 1068B  
Part 1272B

*For storage requirements and expiration date refer to individual bottle labels.*

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**APPLICATION:**

Newcomer Supply Eosin-Nigrosin Stain for Sperm Vitality procedure provides ready-to-use solutions for permanent slide preparation for evaluation of sperm vitality. The Nigrosin Stain provides a dark background for easier recognition of both viable (membrane-intact) and non-viable (membrane-damaged) spermatozoa that will be unstained or stained with the Eosin Y solution.

**METHOD:**

**Solutions:** All solutions are manufactured by Newcomer Supply, Inc.

**STAINING PROCEDURE:**

1. Assess sperm vitality as soon as possible after liquefaction of the semen sample; approximately 30 minutes after collection.
  - a. See Procedure Note #1.
2. Pre-label microscopic slide(s) with appropriate patient identifiers.
3. Mix fresh sample with thorough swirling before pipetting aliquots for staining and evaluation.
4. Mix one drop of fresh semen with two drops of Eosin Y Stain 0.5%, Aqueous NaCl; wait for 30 seconds.
  - a. See Procedure Notes #2 and #3.
5. After 30 seconds, add three drops of Nigrosin Stain 10%, Aqueous; gently swirl to mix.
6. Immediately place one drop of mixture on pre-labeled microscopic slide.
7. Prepare a blood film type smear with a focus on uniform distribution.
  - a. Repeat with remaining mixture for as many smears as needed or requested.
8. Allow slide(s) to air-dry.
9. Coverslip air-dried slides with compatible mounting medium.
10. Examine under oil immersion with x100 objective.

**PROCEDURE NOTES:**

1. There is a 30 minute to 1 hour window from the time of collection in which slide preparation for sperm vitality assessment should occur to avoid the possibility of compromising the sample.
2. Separate droppers or pipettes should be used for each solution and step to avoid any possibility of sample contamination.
3. A small vial, centrifuge tube, well plate, glass slide or other item convenient to the laboratory can be used for mixing purposes.

**RESULTS:**

Viable sperm	White/faint pink heads
Non-viable sperm	Red/dark pink heads
Background	Dark

**REFERENCES:**

1. Bjorndahl, L., I. Soderlund, and U. Kvist. "Evaluation of the One-Step Eosin-Nigrosin Staining Technique for Human Sperm Vitality Assessment." *Human Reproduction* 18.4 (2003): 813-816.
2. Carrell, Douglas T., and Kenneth I. Aston. *Spermatogenesis Methods and Protocols, Methods in Molecular Biology*. Vol. 927. New York: Humana Press, 2013. 13-19.
3. Shambayati, Behdad. *Cytopathology*. Oxford: Oxford University Press, 2011. 332-333.
4. *WHO Laboratory Manual for the Examination and Processing of Human Semen*. 5th ed. Geneva: World Health Organization, 2010. 26-32.
5. Modifications developed by Newcomer Supply Laboratory.