

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.

APPLICATION:

Newcomer Supply Eosin-Nigrosin Stain for Sperm Vitality procedure provides ready-to-use solutions for permanent slide preparation and 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. Mix sample with thorough swirling before aliquoting.
3. In a small vial, centrifuge tube, well plate or slide, combine;
 - a. Fresh semen One drop
 - b. Eosin Y Stain 0.5%, Aqueous NaCl Two drops
 - c. Wait 30 seconds
 - d. Add Nigrosin Stain 10%, Aqueous Three drops
 - e. Gently swirl to mix
 - f. See Procedure Note #2.
4. Place one drop of mixture on microscopic slide(s) and prepare a film type smear.
 - a. Repeat for as many smears needed.
5. Allow slide(s) to air-dry.
6. Coverslip air-dried slides with compatible mounting medium.
7. Examine microscopically.

PROCEDURE NOTES:

1. There is a 30 to 60 minute window from time of collection in which slide preparation for sperm vitality assessment should occur to avoid compromising the sample.
2. Use separate droppers/pipettes for each solution and step to avoid sample and reagent contamination.

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.