

Sperm classification

Count a minimum of 200 sperm per sample following this criteria:

- **Spermatozoa without dna fragmentation:**
 - Spermatozoa with big halo: those whose halo width is similar or higher than the diameter of the core (Fig. 1).
 - Spermatozoa with medium-sized halo: their halo size is between those with large and with very small halo (Figure 2).
- **"others":** cell nucleid which do not correspond to spermatozoa. One of the morphological characteristics which distinguish them is the absence of tail. These cells must not be included in the estimation of the frequency of sperm with fragmented DNA.
- **Spermatozoa with fragmented dna:**
 - Spermatozoa with small halo: the halo width is similar or smaller than 1/3 of the diameter of the core (Fig. 3).
 - Spermatozoa without halo: (Fig. 4).
 - Spermatozoa without halo and degraded: those that show no halo and present a core irregularly or weakly stained (Fig. 5).

Positive and negative controls (optional)

Positive control: all the sperm cells are shown with halo. Follow the instruction for use, skipping step 7.

Negative control: all the sperm cells are shown without halo. Follow the instruction for use, skipping step 8.

Safety and the environment

- Care should be taken to avoid contact with skin or eyes, and to prevent inhalation. solution (DA) and the lysis solution (LS) contains Work under air removal environment and follow the manufacturer's Material Safety Data regarding safe handling.
- Do not release the products used into the environment. Please follow the specific safety regulation of your laboratory facility with respect to chemicals storage and toxic products disposal as well as the exposure to them.

Precautions

- All patient samples and reagents should be treated as potentially infectious and the user must wear protective gloves, eye protection and laboratory coats when performing the test.
- The test should be discarded in a proper biohazard container after testing.
- Do not eat, drink or smoke in the area where specimens and kit reagents are handled.
- Do not use beyond the expiration date, which appears on the package label.
- The use of gloves and face mask is recommended.

Store conditions

After receiving the kit, store it between 2° and 8°C. and lysis solution between -10° and 4°C preferred freeze

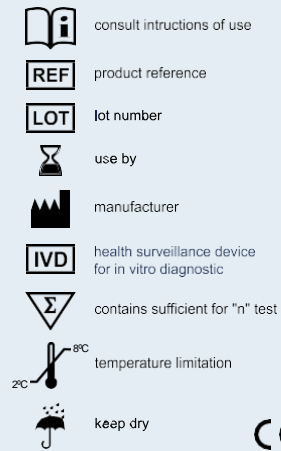


ENO SPERM

DNA Fragmentation Kit

REF EN 12007

for 10 determinations



ENO SPERM DNA has been developed in response to the needs of users of the SCD test (Sperm Chromatin Dispersion test) for assessing sperm DNA fragmentation in human.

Principle of the method

Intact unfixed sperm (fresh, frozen/unthawed, diluted samples) are immersed in an inert agarose microgel on a pretreated slide. An initial DA agent treatment denatures DNA in those sperm cells with fragmented DNA. Following this, the lysis solution removes most of the nuclear proteins, when absence of massive DNA breakage is present, nucleoids from sperm with fragmented DNA either, do not show a dispersion halo or the halo is minimal.

Description of kit reagents

Every kit contains the necessary to perform 10 assays. The components are:

- Agarose Cell Support (ACS); 1 screw tube
- Super-Coated Slides (SCS); 10 units
- Solution 1 (DA) Denaturant Agent, one 10 ml drop bottle
- Solution 2 (LS) Lysis Solution, one 10 ml drop bottle (R1) + (R2) X2 droppers mix one by one before work its valid for 58 days once mixed and remember to keep it freeze.
- Solution 3 (SSA) eosin Staining Solution A, one 10 ml drop bottle
- Solution 4 (SSB) thiazine Staining Solution B, one 10 ml drop bottle
- Float

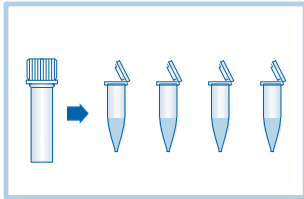
Material and equipment required, not provided with the kit

Bright field or fluorescence microscope, fridge at 4° C, incubation bath (s) at 37° C and 95-100° C, plastic gloves, glass coverslips (24 x 24 mm). Micropipettes, Petri dishes or similar tray, disposable pipettes, Eppendorf, distilled water, ethanol at 70% and 100%. Microwave oven and fume hood.

Sperm sample

Fresh semen samples should be collected in a sterile recipient. The sperm DNA fragmentation assay should be performed immediately once the sperm sample has been obtained or thawed after cryopreservation.

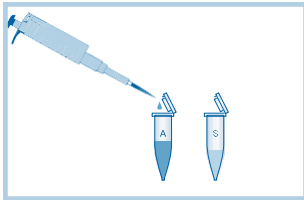
Instructions for use



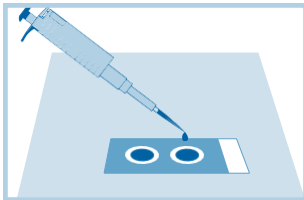
1.
 - 1.1 Place the agarose screw tube (ACS) into the float and melt using a water bath (or a beaker with water on a hot plate) at 95-100°C for 5 minutes or until it is completely melted. Otherwise, if you prefer melting the agarose using a microwave oven, fill 100 ml of water in a beaker. Then, place the ACS slightly opened with the float inside the beaker and heat it at maximum power for 1.5 minutes. Watch constantly and stop the process as soon as the water starts boiling. Please do not keep the ACS boiling inside the microwave! Aliquot 10 Eppendorf tubes (ET) with 100 microliters of the agarose melted. Immediately after, keep the Eppendorf to be used at 37°C for 5 minutes to prevent the gelification.
 - 1.2 The remaining Eppendorf tubes which are not going to be used at that moment will be storage in the fridge along with the kit.
 - 1.3 Set Solutions 1 and 2 at room temperature (22°C) during the whole process.
 - 1.4 Prepare and select the Super-Coated Slides (SCS) which are going to be used.



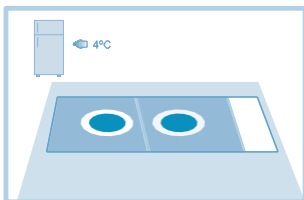
2. Dilute the sperm sample in an appropriate human sperm extender or PBS to a maximum of 20 million sperm per milliliter incubated at 37°C.



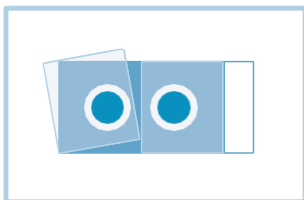
3. Immediately after, transfer 20 µl of the sperm sample to the Eppendorf tube and mix gently with a micropipette. The formation of bubbles shall be prevented.



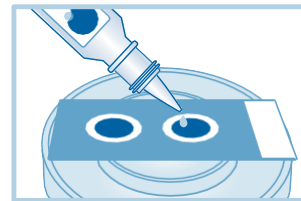
4. Following, place a drop of 10 µl of the cell suspension onto the center of sample well ("S"). Cover with a coverslip. Press gently, avoiding air bubbles formation. Slides must be held in a **horizontal position** throughout the entire process. Use the "C" well to process a control sample.



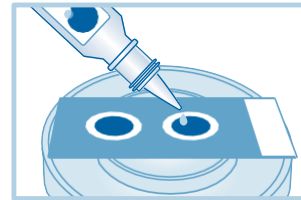
5. Place the slide on a cold surface (for example, a metal or glass plate pre cooled at 4°C) and transfer into the fridge at 4°C, for 5 minutes to solidify the agarose.



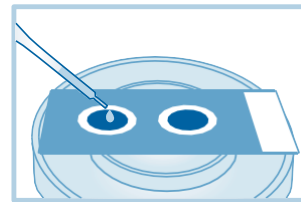
6. Take the slide out of the fridge and remove the coverslip by **sliding it off gently**. All the processing must be performed at room temperature (22 °C)



7. Place the slide horizontally in an elevated position as suggested in the figure into a Petri dish or similar tray. Apply Solution 1 (DA) on the well making sure **it is fully covered by the reactive during the whole process**. for 7 minutes. Then, remove the reactive by tilting until completing the drying and place the slide horizontally in an elevated position as suggested in the figure. **It is very important to remove the reactive without shaking. We will perform the removal by tilting and letting it slide.**

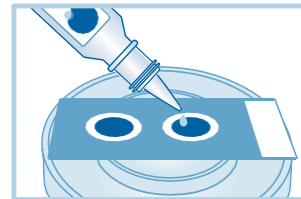


8. Apply Solution 2 (LS) on the well making sure it is fully immersed. for 20 minutes. Then, remove the reactive by tilting until completing the drying and place the slide horizontally in an elevated position as suggested in the figure. It is very important to remove the reactive without shaking. **We will perform the removal by tilting and letting it slide.**



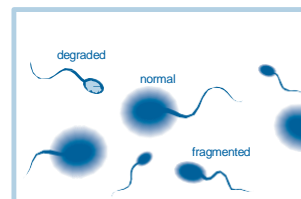
9. Wash the slide for 5 min covering with abundant distilled water and using a disposable pipette. Then, remove the reactive by tilting until completing the drying and place the slide horizontally in an elevated position as suggested in the figure.

Dehydrate by flooding with 70% ethanol, using a disposable pipette for 2 minutes. Drain and apply 100% ethanol for 2 minutes. Drain and allow to dry horizontally on filter paper or similar. After drying, processed slides may be kept in slide boxes at room temperature in a dry and dark place for several months.



10. Place the slide horizontally in an elevated position into a Petri dish or similar tray. Apply Solution 3 (SSA) on the wells making sure these are fully immersed for 7 minutes. Then, remove the stain by tilting until completing the drying and place the slide horizontally in an elevated position.

11. Apply Solution 4 (SSB) on the wells making sure these are fully immersed. for 7 minutes. Then, remove the stain by tilting. Remove the excess of stain and allow to dry at room temperature.



12. Visualize under bright field microscopy. If the staining is too intense; the slide might be washed in tap water. If the staining is too weak, immerse the slide in 100% ethanol, allow to dry and repeat step 10). For fluorescence microscopy staining, please contact the authorized dealer.

$$\text{SDF (\%)} = \frac{\text{Fragmented + degraded}}{\text{Total}} \times 100$$

13. Calculate the percentage of sperm with fragmented DNA. The results should be evaluated taking into account all clinical and laboratory findings related to the sperm sample.