

Sperm classification

Count a minimum of 300 sperm per sample, according to the following criteria:

- **Spermatozoa without DNA fragmentation:**
 - Spermatozoa with big halo: those whose halo width is similar or larger 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 (Fig. 2)
- **"Others":** cell nuclei 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 control

Positive control: all the sperm cells are shown with halo with the exception of degraded spermatozoa. Follow the instruction for use, skipping step 8.

Warnings, environment and 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.

Care should be taken to avoid contact with skin or eyes, and to prevent inhalation. The acid solution (DA) contains Hydrochloric acid, and the lysis solution (LS) contains Dithiothreitol and ECOSURF™. Work under air removal environment and follow the manufacturer's Material Safety Data regarding safe handling.

The test should be discarded in a proper biohazard container after testing. Do not dump into sewers or waterways. 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.

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.














Material Safety Data Sheet is available on request.

Store conditions

After receiving the kit, store it between 2 - 30 °C. Expiry date is on label. After opening the kit is stable for 12 months. 



halosperm®
Kit **REF** HT - HS10
for 10 determinations

	consult instructions of use
	product reference (catalogue number)
	batch number
	serial number
	use-by date
	manufacturer
	date of manufacture
	in vitro diagnostic medical device
	contains sufficient for "n" test
	temperature limit
	keep dry
	attention
	danger



halosperm has been developed by Halotech DNA in response to the needs of users of the SCD test (Sperm Chromatin Dispersion test) for assessing sperm DNA fragmentation in human. IVD for professional use only.

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 acid treatment denatures DNA in those sperm cells with fragmented DNA. Following this, the lysis solution removes most of the nuclear proteins. When massive DNA breakage is present, nucleoids from sperm with fragmented DNA do not show a dispersion halo or the halo is minimal. This test is an aid in the diagnosis. Interpretation of the results will be under medical criteria.

Performance characteristics

Sensitivity	93.0 %
Specificity	93.0 %
Repeatability	93.4 %
Reproducibility	94.0 %
Trueness	91.6 %
Accuracy	97.1 %
Interferent substances	-
Cut-off value ^[1]	IUI: SDF ≤ 20 % ; IVF/ICSI: SDF ≤ 25 %

[1] Esteves SC et al., Andrologia 2021 53(2):e13874. doi: 10.1111/and.13874.

Description of kit components

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

- (SCS) Super-Coated Slides, 10 units
- (ACS) Agarose Cell Support, 10 units
- Solution 1 (DA) Denaturant Agent, 1mL
- Solution 2 (LS) Lysis Solution, 110 ml
- (HSF) Float, 1 unit
- Instructions For Use

Material and equipment required not provided with the kit

Bright field or fluorescence microscope, fridge at 2-8 °C, water bath (s) at 37 °C and 95-100 °C, PVC (polyvinyl chloride) gloves, glass coverslips (24 x 24 mm). Micropipettes, incubation tray (Halotech, HT-BAN) or similar tray, disposable pipettes, filter paper or similar, distilled water, ethanol at 70% and 100%. Microwave, oven and fume hood. Recommended solution for microscopy visualization:

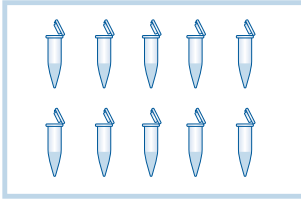
- Bright field microscopy: Brightfield Staining Solution (Halotech, HT-BFS)
- Fluorescence microscopy: Fluorochromes for DNA staining. **fluored®** (Halotech, HT-RFS100) and **fluogreen®** (Halotech, HT-GFS100)
- Phosphate buffer saline (PBS).

Beware that all equipment is calibrated.

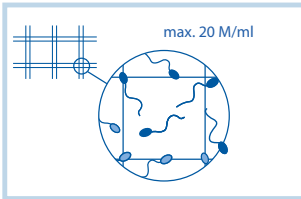
Sperm sample

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.

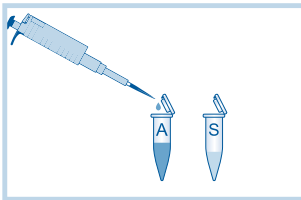
Instructions for use



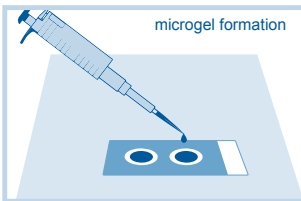
1.
 - 1.1 Place the agarose Eppendorf tube (ACS) into the float and melt using a water bath (or a beaker with water on a hot plate) at 95-100 °C for 5 minutes or until it is completely melted. Otherwise, if you prefer melting the agarose using a microwave oven, fill 100 ml of water in a beaker. Then, place the ACS 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!** 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 stored in the fridge along with the kit.
 - 1.3 Set Lysis Solution at room temperature (22 -25 °C, approx.) during the whole process.
 - 1.4 Prepare and select the Super-Coated Slides (SCS) which are going to be used.



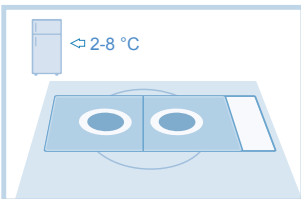
2. Dilute the sperm sample in an appropriate human sperm extender or PBS to a maximum of 20 million sperm per ml.



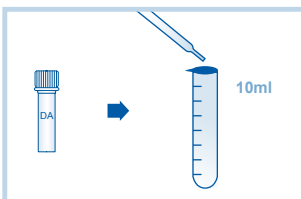
3. Immediately after, transfer 50 µl of the sperm sample to the Agarose tube (ACS) and mix gently with a micropipette. The formation of bubbles shall be prevented.



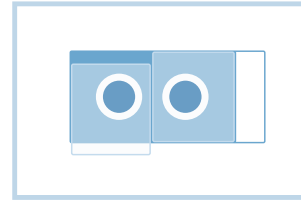
4. Following, place a drop of 8 µl of the cell suspension onto the center of sample well ("S"). Cover with a coverslip. Press gently, avoiding air bubbles formation. Slides must be held in a **horizontal position** throughout the entire process. Use the "C" well to process a control sample.



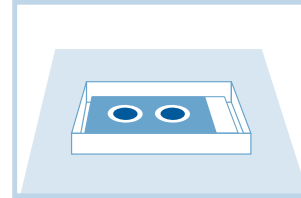
5. Place the slide on a cold surface (for example, a metal or glass plate pre-cooled at 2-8 °C) and transfer into the fridge at 2-8 °C, for 5 minutes to solidify the agarose.



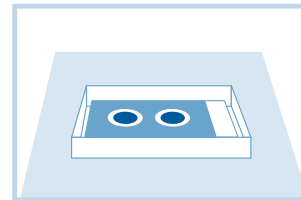
6. Prepare the denaturant solution (DA). To do this, add 80 µl of the contents of the acid denaturation solution (Tube labelled DA) to 10 ml of distilled water, mix and place in the incubation tray.



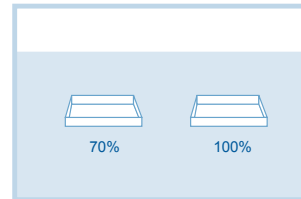
7. Take the slide out of the fridge and remove the coverslip by sliding it off gently. All the processing must be performed at room temperature (22-25 °C approx.)



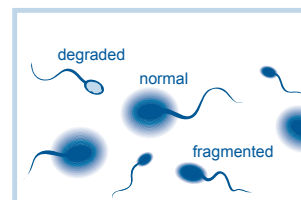
8. Immediately, immerse the slide into the acid denaturation solution from step 6 in a horizontal position, incubate for 7 minutes. **Do not exceed the scheduled time! Beware that it is fully covered by the reagent during the whole process.**



9. Afterwards, place it in another incubation tray containing 10 ml of tempered lysis solution (LS). Incubate for 25 minutes in horizontal position.



10. Pick the slide up and set up horizontally into a tray containing abundant distilled water in order to wash the lysis solution. Incubate for 5 minutes.



12. Visualize under bright field or fluorescence microscopy depending on the type of staining. If the staining is not appropriate, contact your supplier.

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

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