

## Sperm classification

Count a minimum of 300 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 (Big halo).
- Spermatozoa with medium-sized halo: their halo size is between those with large and with very small halo (Medium halo)

"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 (Small halo).
- Spermatozoa without halo: (Without halo).
- Spermatozoa without halo and degraded: those that show no halo and present a core irregularly or weakly stained (Degraded).

## Positive and negative controls

- **Positive control:** all the sperm cells are shown with halo. Follow the instruction for use, skipping step 8.
- **Negative control:** all the sperm cells are shown without halo. Follow the instruction for use, skipping step 9.

## Safety and the environment

- 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 Triton X-100. 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.
- Material Safety Data Sheet is available on request.

## Store conditions

After receiving the kit, store it between 2°C and 30°C and protected from the light  
After opening the kit is stable for 12 months



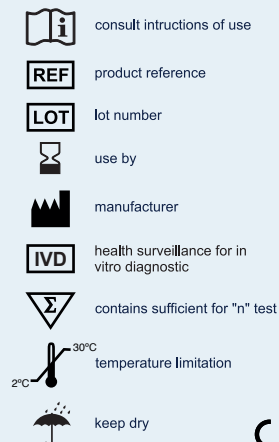
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halosperm®

Kit REF HT - HS10  
for 10 determinations



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.

## Principle of the method

Intact unfixed sperm (fresh, frozen/unthawed, diluted samples) are immersed in an inert agarose microgel on a pretreated slied. 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 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. **The diagnostic sensitivity and specificity is 93%.** This test is an aid in the diagnosis. Interpretation of the results will be under medical criteria.

## Description of kit reagents

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

- Super-Coated Slides (SCS); 10 units
- Eppendorf Agarose Tubes (ACS); 10 units
- (DA) Denaturant Agent 1mL screw tube
- (LS) Lysis Solution, one 110 ml bottle
- Float
- Instructions For Use

## 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, Incubation tray (Halotech, HT-BAN) or similar tray, disposable pipettes, distilled water, etanol at 70% and 100%. Microwave oven and fume hood.  
Recommended solution for microscopy visualization:

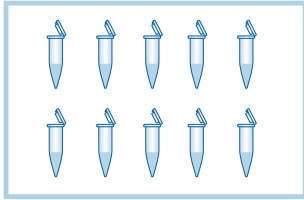
- Bright field microscopy: Brightfield Staining Solution (Halotech, HT-BFS)
- Fluorescence microscopy: Fluorochromes for DNA staining. FluoRed (Halotech, HT-RF100) and FluoGreen (Halotech, HT-GF100)
- Phosphate buffer solution pH 6.88 (Merck 1.07294.1000).
- Mounting medium: Eukitt® (Panreac 253681 ).

**Be ware 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



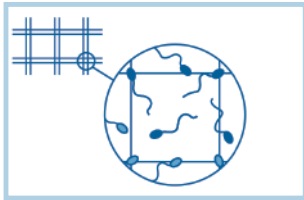
### 1.

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 storage in the fridge along with the kit.

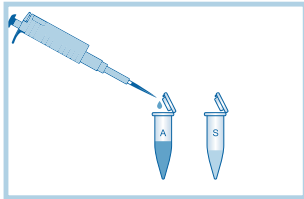
1.3 Set Lysis Solution at room temperature (22°C aprox.) during the whole process.

1.4 Prepare and select the Super-Coated Slides (SCS) which are going to be used.



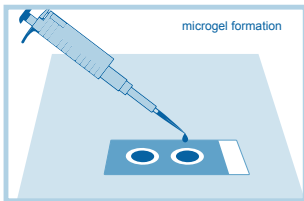
### 2.

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



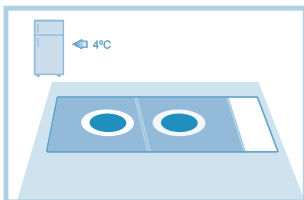
### 3.

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



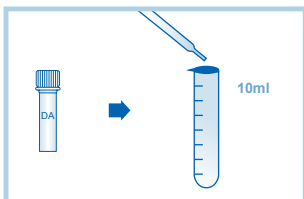
### 4.

Following, place a drop of 8 µl of the cell suspension onto the centre 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.



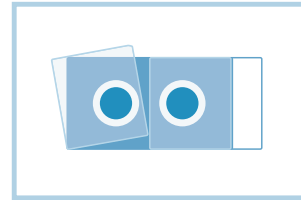
### 5.

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.



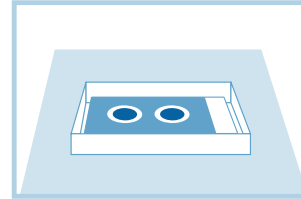
### 6.

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



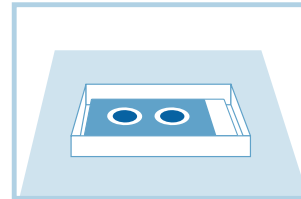
### 7.

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 °C aprox.)



### 8.

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

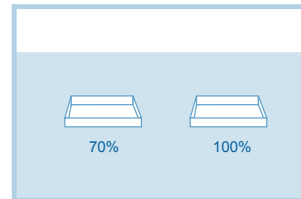


### 9.

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

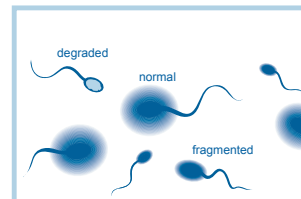
### 10.

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



### 11.

Pick the slide up and place it horizontally into a tray with 70% ethanol (2 minutes) and 100% ethanol (2 minutes). Leave to dry at room temperature in a horizontal position on filter paper or similar. After drying, the processed slides may be kept in archive boxes at room temperature, in the dark, for months. After this, the staining kit can be applied following the instructions of your supplier.



### 12.

Visualize under bright field microscopy or fluorescence. 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.

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

### 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. Thresholds for frequency of Sperm DNA Fragmentation (SDF) have been suggested by Dr. Evenson et al. (Evenson and Nixon, Reprod Biomed Online 12:466-472, 2006).