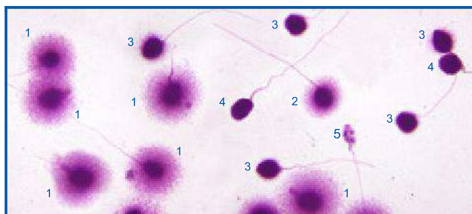
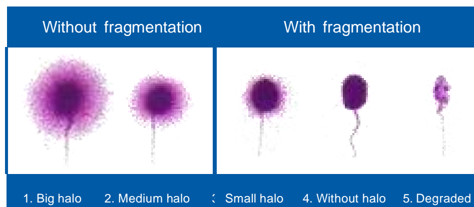




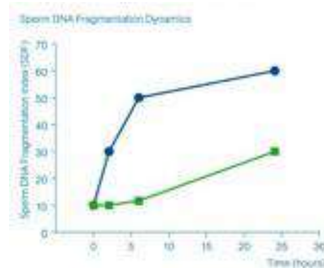
Sperm classification

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).

Evaluating the results

The blue line in the graph represents an individual with a fast increase in DNA fragmentation dynamics within the first hours of incubation. On the contrary, the green line represents an individual with stable DNA fragmentation dynamic. The profile of the curve should be considered as an additional parameter for semen quality, useful to manage properly samples in the laboratory and program different types of ART.



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 - 8 ºC. Expiry date is on label.
After opening the kit is stable for 9 months.

halosperm® is a trademark by halotech DNA, S.L.
C/FARADAY 7, 1º PLANTA CAMPUS DE CANTOBLANCO – 28049 MADRID SPAIN
Tel: + 34 91 279 69 50 / www.halotechdna.com / info@halotechdna.com

Version 05 / 2023

dyn-halosperm®

Kit REF HT - DHS5G2
for 5 determinations

Symbol	Meaning
i	consult instructions of use
REF	product reference (catalogue number)
LOT	batch number
SN	serial number
	use-by date
	manufacturer
	date of manufacturer
IVD	in vitro diagnostic medical device
Σ	contains sufficient for "n" test
	temperature limit
	keep dry
!	attention
	danger

dyn-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 dynamics in human spermatozoa.
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 no massive DNA breakage is present, nucleoids with large haloes of spreading DNA loops, emerging from a central core, are produced. Nucleoids from sperm with fragmented DNA do not show a dispersion halo or the halo is minimal.

Performance characteristics

Sensitivity	93.0 %
Specificity	93.0 %
Repeatability	93.4 %
Reproducibility	94.0 %
Trueness	91.6 %
Accuracy	97.1 %
Interferent substances	-

Description of kit components

Every kit contains the necessary to perform 5 assays. The components are:

- Agarose Cell Support (ACS); 2 screw tubes
- Super-Coated Slides (SCS); 5 units
- Eppendorf Tubes (ET); 5 units
- Coloured tubes (CT); 20 units (4 colours)
- Solution 1 (DA) Denaturant Agent, 10 ml drop bottle
- Solution 2 (LS) Lysis Solution, 10 ml drop bottle
- Solution 3 (SSA) Staining Solution A, 10 ml drop bottle
- Solution 4 (SSB) Staining Solution B, 10 ml drop bottle
- (HSF) Float, 1 unit
- Instructions for Use

Material and equipment required not provided with the kit

Bright field microscope, fridge at 2-8 ºC, water bath (s) at 37 ºC and 95-100 ºC, PVC (polyvinyl chloride) gloves, glass coverslips (24 x 50 mm). Micropipettes, Petri dishes or similar tray, disposable pipettes, distilled water, microwave oven, filter paper or similar, ethanol at 70% and 100%. Phosphate buffer saline (PBS).
Beware that all equipment is calibrated.

Sperm sample

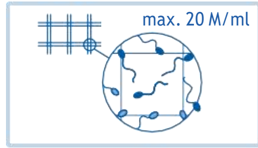
Fresh semen samples should be collected in a sterile container. The sperm DNA fragmentation assay should be performed immediately after thawing and the processing times of the aliquots should be carefully recorded so that the results may be accurately represented in a graph.

Instructions for use

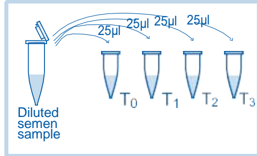
The analysis of sperm DNA fragmentation dynamics is a process that basically recreates the effect of time on DNA quality when a sperm sample is incubated with an oocyte. This is similar to what occurs during IVF or IUI. That is, a semen sample is prepared for use and incubated at 37 °C in an incubator or water bath.
Two-time frames are recommended depending on how the resulting information is used in specific ART cycles.

Short: 0 minutes (T_0), 1 hour (T1), 2 hours (T2), 6 hours (T3). **Long:** 0 minutes (T_0), 2 hour (T1), 6 hours (T2), 24 hours (T3).

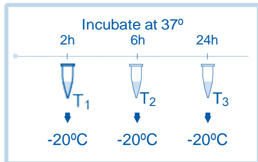
Sperm sample preparation



1.
Dilute the sperm sample in an appropriate human sperm extender or PBS to a maximum of 20 million sperms per ml.

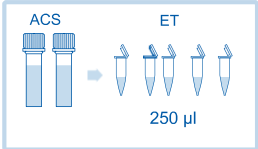


2.
Aliquot 25 µl of semen into each of the coloured Eppendorf tubes provided in the kitand label them as T_0, T_1, T_2 and T_3.

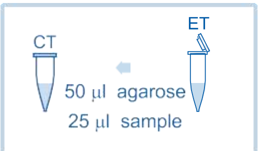


3.
Freeze the T0 sample at -20 ºC immediately (no cryopreservant is necessary). Incubate the three remaining aliquots in an incubator at 37 ºC.
Remove the aliquots from the incubator at the chosen times and freeze at -20 ºC. The sperm DNA fragmentation values of frozen samples do not vary if these are analysed rapidly alter thawing.

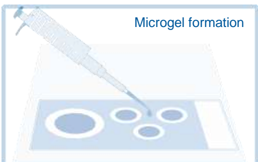
Inclusion of sperm sample in agarose microgel



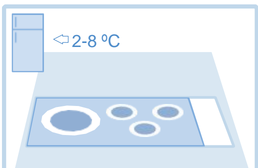
4.
4.1 Place the agarose screw tubes (ACS) into the float and melt using a water bath at 95-100 ºC for 5 minutes until it is completely melted. Otherwise, if you prefer melting the agarose using a microwave oven, fill 100 ml of water in a beaker. Then, place the ACS slightly opened with the float inside 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 5 Eppendorf tubes with 250 µl of the agarose melted. Use one ET per slide. Immediately after, keep the Eppendorf to be used at 37 ºC for 5 minutes to prevent the gelification.
4.2 The remaining Eppendorf tubes which are not going to be used at that moment will be stored in the fridge along with the kit.
4.3 Set Solutions 1 and 2 at room temperature (22-25 °C approx.) during the whole process.



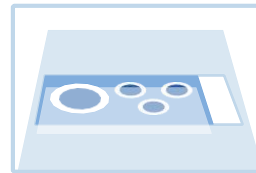
5.
Meanwhile, thaw the frozen samples at room temperature for 3-5 minutes. Transfer 50 µl of liquid agarose to each thawed (coloured) tube and mix gently. Maintain the tubes at 37 °C.



6.
Place 6 µl from the T_0 cell suspension to the T_0 well on the supercoated slide. In the same way, transfer 2 µl of mixtures from the T_1, T_2 and T_3 tubes onto the corresponding wells on the super-coated slide. Immediately afterwards place a glass 24 x 50 mm coverslip on top of the sample and press gently, avoiding air bubbles formation.
Slides must be held in a horizontal position throughout the entire process.

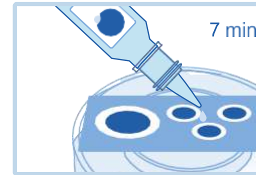


7.
Place the slide on a cold surface (for example, a metal or glass plate pre-cooled at 2-8 ºC) and transfer into the fridge at 2-8 ºC, for 5 minutes to solidify the agarose.



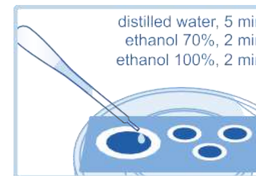
8.
Take the slide out of the fridge and remove the coverslip by sliding it off gently. All the processing must be performed at room temperature (22-25 ºC approx.).

Sperm sample processing



9.
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 reagent during the whole process**. Incubate for 7 minutes. Then, remove the reagent by tilting and place the slide horizontally in an elevated position as suggested in the figure. **It is very important to remove the reagent without shaking. We will perform the removal by tilting and letting it slide.**

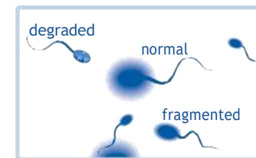
10.
Apply Solution 2 (LS) on the well making sure it is fully immersed. Incubate for 20 minutes. Then, remove the reagent by tilting and place the slide horizontally in an elevated position as suggested in the figure. It is very important to remove the reagent without shaking.
We will perform the removal by tilting and letting it slide.



11.
Wash the slide for 5 minutes covering with abundant distilled water and using a disposable pipette. Then, remove the water by tilting and place the slide horizontally in an elevated position as suggested in the figure. Dehydrate by flooding with 70% ethanol, using a disposable pipette and incubate for 2 minutes.
Drain and apply 100% ethanol for 2 minutes. Drain and allow to dry 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.

Staining and visualization

12.
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. Incubate for 7 minutes. Then, remove the stain by tilting and place the slide horizontally in an elevated position.
Apply Solution 4 (SSB) on the wells making sure these are fully immersed. Incubate for 7 minutes. Then, remove the stain by tilting. Remove the excess of stain and allow to dry at room temperature.



13.
Visualize under bright field microscopy. If the staining is too intense, the slide may be washed with tap water. If the staining is too weak, immerse the slide in 100% ethanol, allow to dry and repeat step 12. For fluorescence microscopy staining, please contact the authorized dealer.

Interpreting the results

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

$$\text{Sperm DNA fragmentation rate} = \frac{(T_h - T_0)}{h}$$

h: incubation time

14.
Calculate the percentage of sperm with fragmented DNA. The results should be evaluated considering all clinical and laboratory findings related to the sperm sample.

For future assays

!

Use as many Eppendorf tubes (ET with aliquoted agarose) as semen samples are going to be evaluated. Place the ET into the float and melt using a water bath (or a beaker with water on a hot plate) at 95-100 ºC 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 ET 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. Continue the protocol from step 4.3.
Please do not keep the ACS boiling inside the microwave!