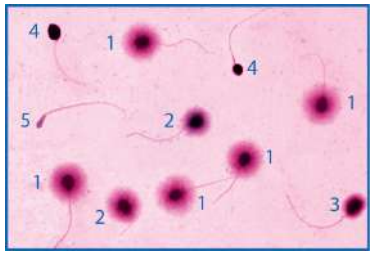
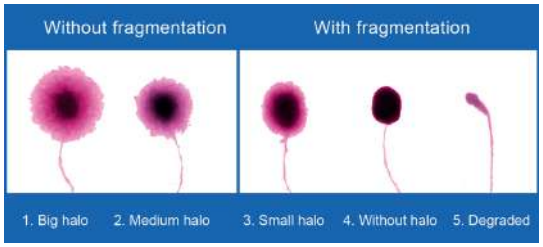
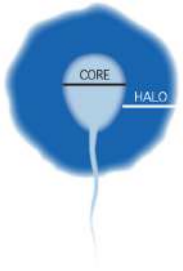




Sperm classification

Count a minimum of 300 sperm per sample, following these criteria:

- Sperm without DNA fragmentation:
  - Sperm with a large halo: halo width equal to or greater than the diameter of the core (Fig. 1).
  - Sperm with a medium-sized halo: halo size between large and small halo (Fig. 2).
- Sperm with DNA fragmentation:
  - Sperm with a small halo: halo width equal to or smaller than 1/3 of the diameter of the core (Fig. 3).
  - Sperm without ahalo (Fig. 4).
  - Degraded sperm: no halo present and core irregularly or weakly stained (Fig. 5).
- "Others": cell nuclei which do not correspond to sperm. A distinguishing morphological characteristic is the absence of a tail. These cells should not be included in the estimation of the frequency of sperm with DNA fragmentation.



Postive control

All sperm cells present a halo, except for degraded spermatozoa. To perform the positive control use the 'C' well following the instructions for use, skipping step 7.

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.

!

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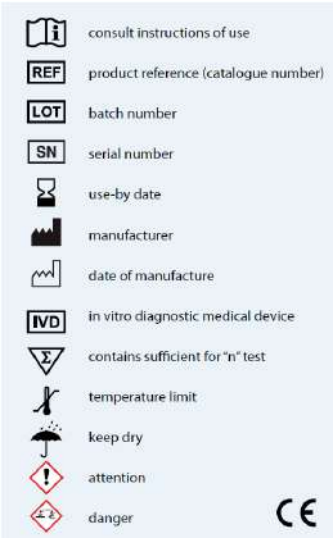
C/FARADAY 7, 1º PLANTA CAMPUS DE CANTOBLANCO – 28049 MADRID SPAIN
Tel: + 34 91 279 69 50 / www.halotechdna.com / info@halotechdna.com

Version 02/2025

halosperm®G2 PRO

Kit REF HT- HSG2P
for 10 determinations



halosperm® G2 PRO is the new alternative offered by Halotech DNA to address the need for a quicker SCD (Sperm Chromatin Dispersion) test to assess sperm DNA fragmentation in humans.

IVD for professional use only.

Principle of the method

Sperm samples (fresh, diluted, or frozen/unthawed) are embedded in an inert agarose microgel on a pretreated slide. An initial acid treatment denatures the DNA in sperm cells with fragmented DNA. Following this, the lysis solution removes most of the nuclear proteins. If the sperm exhibit extensive DNA breakage, they will not show a dispersion halo, or it will be minimal. This test is an aid in diagnosis. The interpretation of the results will be based on 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:

- (ACS) Agarose Cell Support; 1 screw tube
- (SCS) Super-Coated Slides; 10 units
- (ET) Eppendorf Tubes; 10 units
- Solution 1 (DA) Denaturant Agent; 10 mL
- Solution 2 (LS) Lysis Solution; 10 mL
- Solution 3 (SSA) Staining Solution A; 10 mL
- Solution 4 (SSB) Staining Solution B; 10 mL
- (HSF) Float; 1 unit
- Instructions for Use

Material and equipment required not provided with the kit

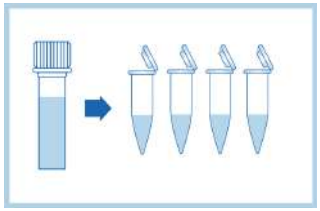
Bright field microscope, fridge at 2-8 ºC, water bath (s) at 37 ºC and 95-100 ºC, PVC (polyvinyl chloride) gloves, glass coverslips (24 x 24 mm). Micropipettes, Petri dishes or similar tray, disposable pipettes, distilled water, phosphate buffer saline (PBS), microwave oven, filter paper or similar, 96% ethanol at 2-8 ºC.
Beware that all equipment is calibrated.

Sperm sample

The fresh semen sample should be collected in a sterile container. The sperm DNA fragmentation assay should be performed immediately after the sperm sample is received or thawed.

Instructions for use

Please note: ethanol at 96% must be at 2-8 ºC until use.



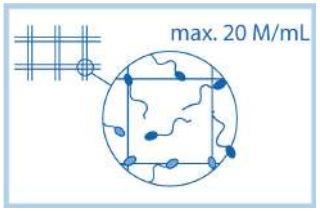
1.

1.1 Allow Solutions 1 and 2 to warm to room temperature (22-25 ºC approx.).

1.2 Place the Agarose Cell Support (ACS) in the float and melt using a water bath at 95-100 ºC for 5 minutes or until fully melted. If preferred, the agarose can be melted in a microwave. To do this, fill a beaker with 100 mL of water, place the ACS with the float inside, and heat at maximum power for 1.5 minutes. Monitor constantly and stop the process as soon as the water begins to boil. **Please, do not keep the ACS in the boiling water.** Aliquot 10 Eppendorf tubes (ET) with 50 µL of the melted agarose. Immediately afterwards, keep the ETs at 37 ºC to prevent gelation.

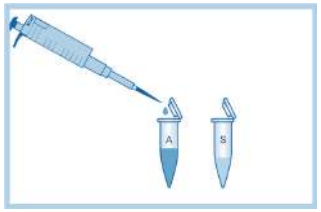
1.3 The remaining ETs that are not going to be used at that moment should be stored in the fridge along with the kit.

1.4 Prepare the Super-Coated Slides (SCS) that are to be used.



2.

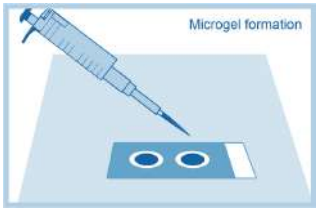
Dilute the sperm sample in an appropriate human sperm extender or PBS to a maximum concentration of 20 million sperm per mL.



3.

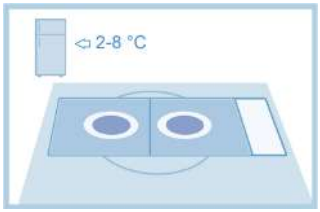
Immediately afterwards, transfer 25 µL of the sperm sample to the ET and mix gently with a micropipette. The formation of bubbles should be avoided.

Sperm sample processing



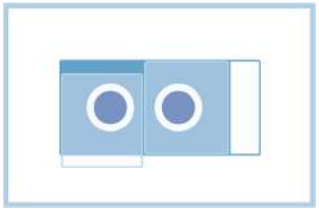
4.

Next, place 8 µL of the mixture onto the center of well 'S'. Cover with a coverslip and press gently. The slides must be kept in a horizontal position throughout the entire process. Repeat the procedure using well 'C' to perform the positive control.



5.

Place the slide on a cold surface (e.g., a metal or glass plate pre-cooled to 2-8 ºC) and then place it in the fridge at 2-8 ºC for 2 minutes.



6.

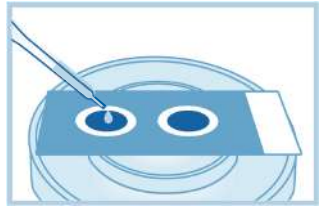
Take the slide out of the fridge and remove the coverslip by gently sliding it off. All processing must be performed at room temperature (22-25 ºC approx.).

7.

Place the slide horizontally in an elevated position as shown in the figure and apply Solution 1 (DA) to the 'S' well, ensuring it is fully covered by the reagent. Incubate for 1 minute. Then, remove the reagent by tilting the slide and allowing it to slide off; do not remove by shaking.

8.

Apply Solution 2 (LS) to both wells, ensuring they are fully covered. Incubate for 2 minutes. Then, remove the reagent by tilting the slide and allowing it to slide off.



9.

Using a disposable pipette, apply distilled water to fully cover the slide. Then, remove the water by tilting the slide and letting it slide off.

10.

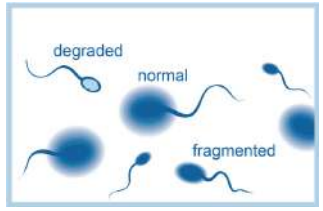
Apply 96% ethanol at 2-8 ºC to the wells, ensuring they are fully covered. Incubate for 2 minutes. Drain the reagent and let the slide dry completely on filter paper or a similar surface. After drying, processed slides may be stored in slide boxes at room temperature for several months before applying the stain.

11.

Place the slide horizontally in an elevated position as shown in the figure. Apply Solution 3 (SSA) to the wells, ensuring they are fully immersed. Incubate for 1.5 minutes. Drain by tilting.

12.

Apply Solution 4 (SSB) to the wells, ensuring they are fully covered. Incubate for 1.5 minutes. Remove the stain by tilting and wash the slide under running tap water, ensuring water flows from the back side of the slide until it runs clear. Allow the slide to dry at room temperature on filter paper or a similar surface.



13.

Visualize under bright field microscopy.

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

14.

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

For future assays

Use as many ETs (with aliquoted agarose) as there are semen samples to be evaluated. Place the ETs into the float and melt using a water bath at 95-100 ºC until the agarose is completely melted. Alternatively, if you prefer to melt the agarose using a microwave oven, fill a beaker with 100 mL of water. Then, place the ET with the float inside the beaker and heat it at maximum power. Monitor constantly and stop the process as soon as the water starts boiling. **Please do not keep the ACS boiling inside the microwave!** Follow the protocol, skipping steps 1.2 and 1.3.