



halosperm® G2 PRO

KIT REF: HT-HSG2P

VERSION: 02

1. INTENDED PURPOSE

Kit for measurement of DNA fragmentation level of spermatozoa as an aid to improve ART outcome associated to male factor. Quantitative and not automated test for semen samples

2. 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

3. 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	IUI: SDF ≤ 20 % ; IVF/ICSI: SDF ≤ 25 %

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

4. 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 units
- Instructions for Use

5. STORE CONDITIONS

After receiving the kit, store it between 2 - 8 °C. Expiry date is on label. After opening, the kit is stable for 9 months.

6. MATERIAL AND EQUIPMENT REQUIRED BUT NOT PROVIDED WITH THE KIT

Bright field 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, Petri dishes or similar tray, disposable pipettes, distilled water, phosphate buffer saline (PBS), microwave oven, filter paper or similar, 96% ethanol at 2-8 °C.

Beware that all equipment is calibrated.

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.

8. 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.

9. TECHNICAL PROCEDURE

Please refer to the current version of the instructions for use for the complete protocol.

10. CONTROL

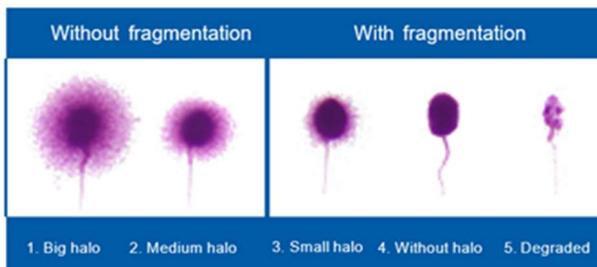
All sperm cells present a halo, except for degraded spermatozoa. Please follow the positive control section of the current version of the instructions for use.

11. RESULTS INTERPRETATION

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).
- 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).
- "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.



12. CUSTOMER SUPPORT

If you have any technical questions or queries regarding use of the kit, please contact support@halotechdna.com

13. REFERENCES

Comhaire, F., Messiaen, A., & Decler, W. (2018). A mathematical model predicting the individual outcome of IVF through sperm-analysis: The role of the HaloSpermG2® DNA fragmentation test. *Medical hypotheses*, 117, 50–53. <https://doi.org/10.1016/j.mehy.2018.05.021>

Liffner, S., Pehrson, I., García-Calvo, L., Nedstrand, E., Zalavary, S., Hammar, M., Rodríguez-Martínez, H., & Álvarez-Rodríguez, M. (2019). Diagnostics of DNA fragmentation in human spermatozoa: Are sperm chromatin structure analysis and sperm chromatin dispersion tests (SCD-HaloSpermG2®) comparable?. *Andrologia*, 51(8), e13316. <https://doi.org/10.1111/and.13316>

14. TECHNICAL MANAGEMENT

Dr. Jaime Gosálvez – Technical Director of Halotech DNA

15. CERTIFICATION AND LICENSE

halosperm® is manufactured under sanitary licence (6893-PS) in accordance with ISO norm 13485:2016 (certificate no. 9124.HDNA). Device complies Directive 98/79/EC and CE mark requirements considering Regulation 2017-746 (art 110) and Regulation 2022-112. 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

16. TABLE OF INTERNATIONAL SYMBOLS

 <p>Consult instructions for use</p>	 <p>Product reference (catalogue number)</p>
 <p>Batch number</p>	 <p>Serial number</p>
 <p>Use-by date</p>	 <p>Manufacturer</p>
 <p>Date of manufacture</p>	 <p>In vitro diagnostic medical device</p>
 <p>Contains sufficient for "n" test</p>	 <p>Temperature limit</p>
 <p>Keep dry</p>	 <p>Attention</p>
 <p>Danger</p>	 <p>CE marking</p>