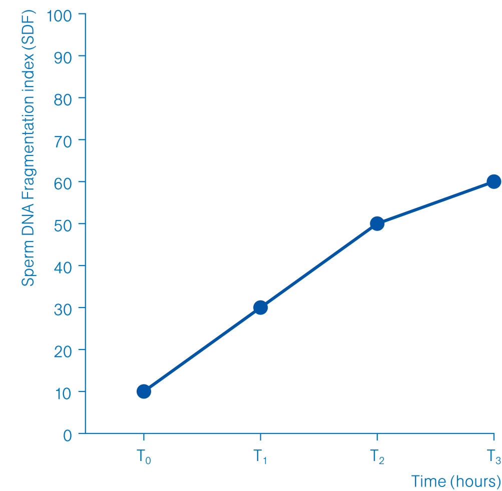
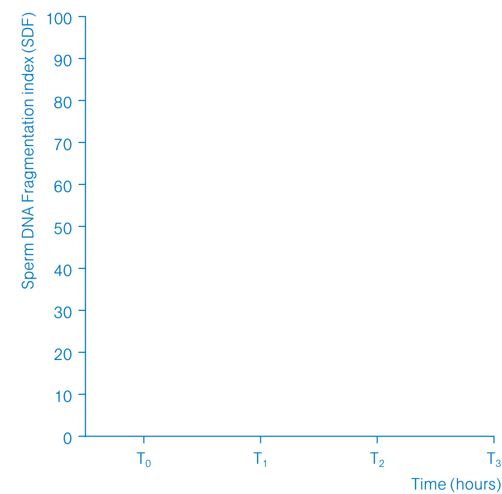
dyn-**halo**sperm®

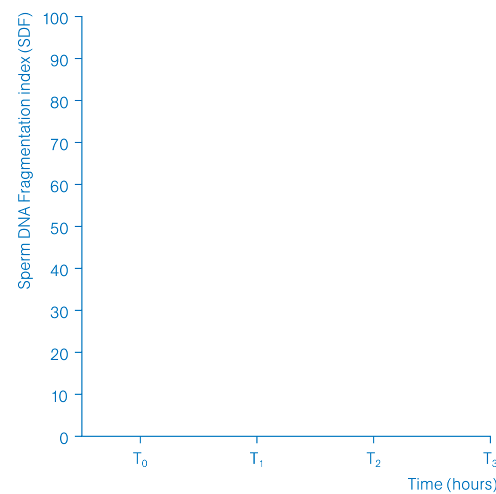
Example



Graph 1



Graph 2



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Halotech Dna, SL
Parque Científico de Madrid / PTM - c/ Santiago Grisolía 2 / 28760 Tres Cantos (Madrid) / T +34 91 571 35 98 / F +34 91 567 15 57 / www.halotech.es / info@halotech.es

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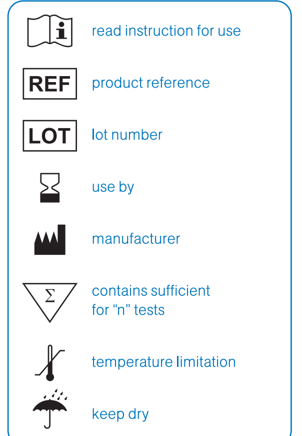
halotech® dna

SAMPLE KIT

dyn-**halo**sperm®

Kit for the study of the dynamics of human sperm DNA fragmentation



applications

Sperm DNA fragmentation values are not stable following ejaculation. The dynamics of DNA fragmentation refers to the measure of the increase of DNA fragmentation over time. **Dyn-Halosperm**® is a new format for the application of the Sperm Chromatin Dispersion (SCD) test that is specifically designed for the study of **DNA fragmentation dynamics** in human spermatozoa.

This version presents various advantages over previous products: (1) a reduced incubation time for the preparations, (2) a reduction in the quantity of reagents used and (3) an overall simplified and more userfriendly format. The redesigned slide with individual wells allows the study of DNA fragmentation dynamics of a patient on a single slide and within only a few minutes.

The SCD test permits the user to easily differentiate between spermatozoa with fragmented DNA from those with intact DNA. This is possible by taking advantage of the differential response of fragmented and intact sperm nuclear chromatin to a treatment of denaturation-deproteination that results in two very distinct patterns of chromatin dispersion. Spermatozoa with intact DNA present a "core" or sperm head surrounded by a halo of dispersed chromatin whereas those with fragmented DNA present no halo.

contents of the kit

- SCS 2 Supercoated slides with 4 wells, corresponding to the time points: basal T_0 (18mm diameter), T_1, T_2 and T_3 (10mm diameter).
- ACS 2 Eppendorf tubes (250μl) with low melting point agarose.
- CT 8 Eppendorf tubes (200 μl; 4 colors) to aliquot the sample at the recommended time points T_0, T_1, T_2 and T_3.
- AD Denaturation Solution (yellow cap).
- LS Lysing Solution (green cap).
- TA Staining Solution A (red cap).
- TB Staining Solution B (blue cap).
- Float.

required materials and equipment not provided in the kit

Bright field microscope, 4°C refrigerator, 37°C temperature-controlled incubator or bath, 95-100°C temperature-controlled incubation bath or microwave oven, 24x50mm glass coverslip, 2-20μl and 10-100μl micropipettes, Petri dish, distilled water, 70% and 100% ethanol, Phosphate buffer solution (PBS) pH 6,88 (Mreck 1,07294,1000) and microscopy mounting medium (Eukitt®, Panreac 253681).

semen sample

Semen samples should be collected in a clean recipient. The assay should be performed as soon as possible and the processing times of the aliquots should be carefully recorded so that the results may be accurately represented in a graph. The analysis of DNA fragmentation dynamics can be performed on either fresh or cryopreserved semen samples.

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 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 (T_1), 2 hours (T_2) and 6 hours (T_3).
- **Long:** 0 minutes (T_0), 2 hour (T_1), 6 hours (T_2) and 24 hours (T_3).

Preparing the sample

1. Equilibrate the Lysing Solution (LS) at room temperature (22°C).
2. Dilute the semen sample in culture medium, semen diluent or PBS, to a final concentration of 16-20 M/ml. Aliquot 25μl of semen into each of the 4 colored Eppendorf tubes provided in the kit and label them as T_0, T_1, T_2 y T_3.
   a. Freeze the T_0 sample at -20°C immediately (no cryoprotectant is necessary).
   b. Incubate the three remaining aliquots in an incubator at 37°C.

Remove the aliquots from the incubator at the chosen times and freeze at -20°C. The sperm DNA fragmentation values of frozen samples do not vary if these are analyzed rapidly after thawing.

3. For every slide to be processed, place an Eppendorf tube containing agarose (250µl) in the float in an incubation bath at 95-100°C for approximately 5 minutes or until the agarose is fully melted. Alternatively, use a microwave oven at maximum power for approximately 2 minutes.
4. Transfer the Eppendorf tube, with the float, to a temperature controlled water bath at 37°C and leave for 5 minutes until the temperature is even.
5. Meanwhile, thaw the frozen samples at room temperature during 5 minutes. Then, transfer the samples to the incubation bath at 37°C for 3 minutes.
6. Transfer 50µl of liquid agarose to each thawed Eppendorf and mix well. Keep the tubes in the 37°C incubation bath.
7. Place 8µl of mixture from the T_0 Eppendorf to the T_0 well on the supercoated slide. In the same way, transfer 4µl of mixture from the the T_1, T_2 and T_3 Eppendorf tubes onto the corresponding wells on the supercoated slide. Immediately afterwards place a glass 24x50mm coverslip on top of the sample and press gently to avoid bubbles. Maintain the preparation in a horizontal position.
8. Put the slide on a cold surface (such as a previously cooled metal or glass plate), and place in the refrigerator at 4°C during 5 minutes, so that the agarose solidifies.

Processing the sample

Note: Always use gloves when the processing steps.

9. Remove the slide from the refrigerator and gently remove the coverslip by sliding it off horizontally. Place the slide on top of the float provided in the kit inside a Petri dish.
10. Apply a few drops of the AD solution over each well making sure they are fully immersed. Incubate for exactly 7 minutes.
11. Drain the solution into the Petri dish by tilting the slide, and carefully apply a few drops of LS solution over each well making sure they are fully immersed. Incubate for 20 minutes.
12. Cover the slide with distilled water and wash for 5 minutes. Drain the water by tilting and replace the slide on top of the float.
13. Dehydrate the sample. To do so, apply 70% ethanol over the slide and incubate for 2 minutes. Drain, apply 100% ethanol and incubate for another 2 minutes.
14. Drain any remaining ethanol and leave to dry.
15. Once the slide is dry, the slide may be kept for several days at room temperature before staining.

Staining and microscopic visualization

Bright filed staining using TA and TB solutions

16. Replace the slide on top of the float inside the Petri dish.
17. Cover the slide with a thin film of staining solution TA, allowing any excess solution to drain into the Petri dish. Incubate for 6 minutes.
18. Without allowing the slide to dry, add a thin film of staining solution TB allowing the excess to overflow into the Petri dish as before. Incubate for 7 minutes.
19. Wash briefly with water and leave to dry.
20. Add a drop of water on the slide and place a coverslip on top to check whether the level of staining is sufficient. If so, dry the slide and mount using Eukitt® mounting solution or equivalent.
    If the stain is insufficiently strong:
    a. Remove coverslip.
    b. Immerse in 100% for 2 minutes.
    c. Dry and repeat staining steps.

Sperm classification

Counting a minimum of 300 spermatozoa per well is recommended for an accurate estimation of the proportion of DNA fragmentation. The spermatozoa can be classified into the following groups.

SPERMATOZOA WITHOUT DNA FRAGMENTATION:

Develop a dispersion halo around the sperm head.

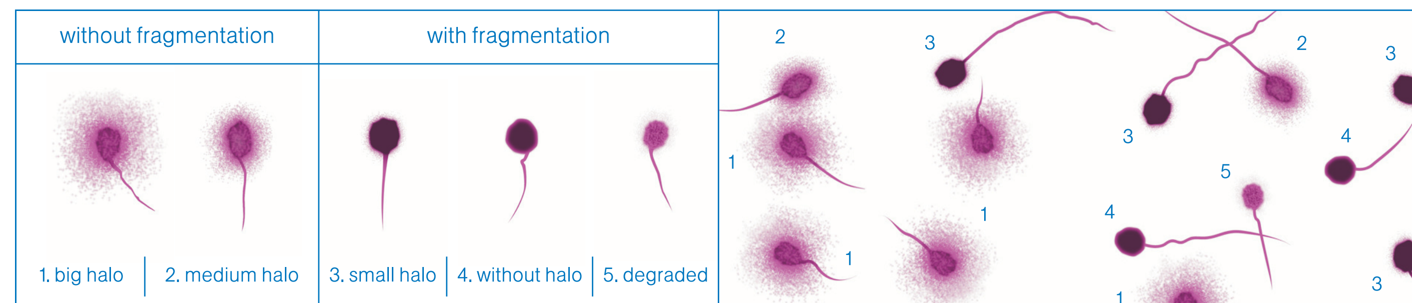
1. Big halo.
2. Medium halo.

SPERMATOZOA WITH FRAGMENTED DNA

Do not develop a halo around the sperm head.

3. Small halo.
4. No halo.
5. Degraded.

20x and 40x objectives are the optimum for visualization and classification of spermatozoa.



Results

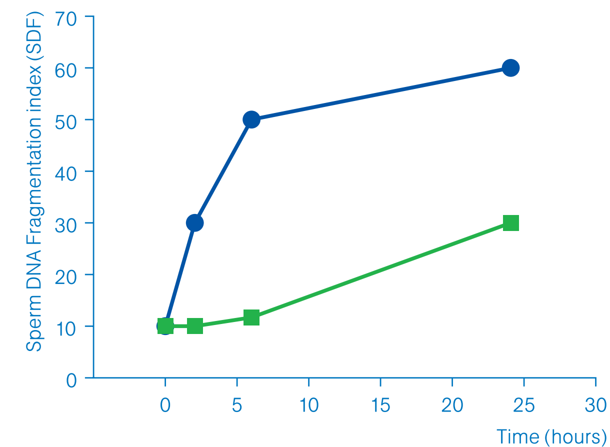
Calculate the Sperm DNA Fragmentation (SDF) level in each well as follows

$$\text{SDF (\%)} = 100 \times \frac{\text{No. of spermatozoa with fragmented DNA}}{\text{No. of spermatozoa counted}}$$

fragmentation	initial evaluation
< 15%	Good
Between 15 and 30%	Medium
> 30%	Critical

Example of a graph of SDF values (%) against time

Sperm DNA Fragmentation Dynamics



Evaluating the results

The blue line in the graph represents an individual with fast increments in DNA fragmentation dynamics within the first hours of incubation. The green line in the graph represents an individual with stable DNA fragmentation dynamics within the first hours of incubation. The profile of the curve of DNA fragmentation dynamics should be considered as an additional parameter for semen quality.

Positive and negative controls

Positive control: sperm cells with halo.

Dilute the semen sample in culture medium, semen diluent or PBS, to a final concentration of 16-20 M/ml. Aliquot 25µl of semen into one empty colored eppendorf tube.
Place an Eppendorf tube containing agarose (250µl) in the float in an incubation bath at 95-100°C for approximately 5 minutes or until the agarose is fully melted.
Alternatively, use a microwave oven at maximum power for approximately 2 minutes.
Transfer 50µl of liquid agarose to each thawed Eppendorf and mix well. Keep the tubes in the 37°C incubation bath.
Transfer 50 µL of a liquid agarose to the colored Eppendorf tube with the sample. Keep the tubes in the 37°C incubation bath.
Place 8µl of mixture from the eppendorf to the T_0 well on the supercoated slide. Follow steps 7 and 8 from the protocol.
Add 50 µl of H_2O_2 (300µM) covering the entire gel surface.
Incubate for 5 minutes in the fridge maintaining the slide in horizontal position. Continue following the protocol as instructed.

Negative control: sperm cells without halo. In one well, follow the instruction for use, omiting step 10.

LIMITATIONS

This test provides quantitative information about basal DNA fragmentation levels in spermatozoa and their rate of change over time. The results of this test should be evaluated taking into account all clinical and laboratory findings related to the sample.

SAFETY AND ENVIRONMENT

Biological samples should be treated as potentially infectious.
Dispose of the products in an appropriate manner following local guidelines for the storage and disposal of chemicals and toxic reagents.

PRECAUTIONS

For profesional use only.

1. All semen samples should be treated as potentially infectious and the user must wear protective gloves, eye protection and laboratory coats when performing the test.
2. The test should be discarded in an appropriate biohazard container after use.
3. Do not eat, drink or smoke in the area where semen samples and kit reagents are handled.
4. Do not use beyond the expiration date which appears on the package label.

STORAGE CONDITIONS

The kit should be stored at room temperature (2-30°C). The reagents have an expiry date of 1 year.