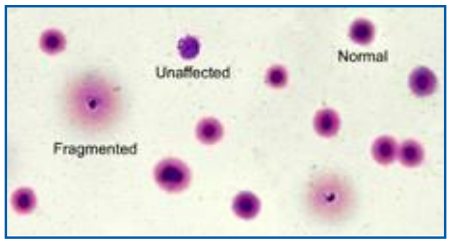

Figure 1: *Cumulus cells* under brightfield microscopy

CCs classification

Count a minimum of 100 CCs per sample according to the following criteria:

Cumulus cells without fragmented DNA:
Cells present a compact chromatin dispersion halo around the core where the diameter of the core and the halo can be equivalent (Figure 1. Normal).

Cumulus cells with fragmented DNA:
Cells present a large, non-compacted and weakly stained chromatin dispersion halo around the core (Figure 1. Fragmented). The halo is larger than the diameter of the core. Highly fragmented cells have reduced cores andminimal haloes of dispersed chromatin.

Unaffected *cumulus cells*:
Cells show no chromatin disruption. These cells should be classified as “unaffected cells” and should be considered to calculate the percentage of DNA fragmentation index (DFI) of the sample (Figure 1. Unaffected).

Safety and the environment

- Do not dispose used products in the environment.
- Follow the center's guidelines for the storage and disposal of toxic substances.
- Biological samples should be handled as potentially infectious and should be disposed according to protocols or procedures of the laboratory / clinic where the samples are processed.

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 on the package label.
- The use of gloves and face mask is recommended.
- Material Safety Data Sheet is available on request.

Store conditions

The kit may be transported at ambient temperature, but once the kit is received, it should be stored between 2-8°C. After opening the kit is stable for 12 months. Expiration date is included in label.

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Version 3 / 2023

halotech®

ovoselect®

Kit REF HT - OVS80
for 80 determinations

	consult instructions of use
REF	product reference
LOT	lot number
	use by
	manufacturer
IVD	medical device for in vitro diagnostic
Σ	contains sufficient for “n” test
	temperature limitation
	keep dry
	warning

CE

ovoselect ® is designed to determine the DNA fragmentation index of *cumulus oophorus cells* (CCs). IVD only for professional use

Principle of the method

Intact unfixed freshly isolated CCs are immersed in an inert agarose microgel on a pretreated slide. The lysis solution removes nuclear proteins and in the absence of DNA damage, cells present a compact chromatin dispersion halo around the core. Cells with DNA damage present a large, non-compact and weakly stained chromatin dispersion halo around the core. The sensitivity is 88% and specificity is 99%. The kit has a trueness of 95.53% and an accuracy of 97.08%. This kit was designed for indirect assessment of oocyte quality. Interpretation of the results will be under medical criteria. No internal control is needed.

Description of kit reagents

Every kit contains the necessary to perform 80 determinations. The components are:

- Agarose Cell Support (ACS); 2 screw tubes
- Super-Coated Slides (SCS); 10 units
- Eppendorf Tubes (ET0.2); 80 units
- Eppendorf Tubes (ET0.5); 10 units
- Solution 1 (LS) Lysis Solution, one 10 ml drop bottle
- Solution 2 (SB) Stabilization Buffer, one 10 ml drop bottle
- Solution 3 (SSA) Eosin Staining Solution A, one 10 ml drop bottle
- Solution 4 (SSB) Thiazine Staining Solution B, one 10 ml drop bottle
- Float

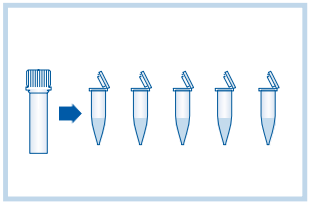
Material and equipment required, not provided with the kit

Brightfield or fluorescence microscope, fridge at 4º C, incubation bath (s) at 37º C and 95-100º C, plastic gloves, glass coverslips (24x24 mm). Micropipettes, Petri dishes or similar tray, disposable pipettes, distilled water, ethanol at 70% and 100%. Microwave oven and fume hood (it is recommended the use of fume hood, but it is not mandatory because of low reagent concentration). Beware that all equipment is calibrated.

CCs sample

Use freshly isolated CCs samples as soon as possible after obtaining the patient's oocytes. The kit can be used in properly cryopreserved CCs. **The slide cannot be processed more than once.**
There are no substances that interfere with the functionality of the kit provided that both the instructions for use of the kit and the standard laboratory procedures for handling CCs are strictly followed.

Instructions for use



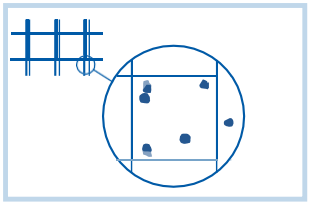
1.

1.1 Place the agarose screw tubes (ACS) into the float and melt using a water bath (or a beaker with water on a hot plate) at 95-100ºC 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. 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 10 Eppendorf tubes (5 for each agarose screw tube) with 180 µl of the melted agarose. Immediately after, keep the Eppendorf to be used at 37ºC for 5 minutes to prevent the gelification.

1.2 The remaining Eppendorf tubes (ET) which are not going to be used at that moment will be stored in the fridge along with the kit.

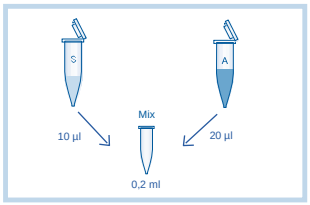
1.3 Set Solutions 1 and 2 at room temperature during the entire process.

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



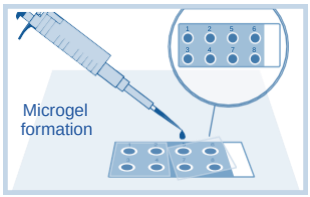
2.

Isolate the CCs from each individual oocyte according to the method routinely used in the laboratory. Each slide is prepared for the analysis of 8 CCs samples isolated from 8 oocytes. Examine under phase contrast microscopy verifying that 10-15 cells per fieldare observed using the 40x objective. If the number of cells is lower than 10, transfer them to an Eppendorf tube or similar and add 0.5-1 ml of PBS or cell culture medium to each tube. Centrifuge at 300xg for 2-5 minutes. Remove the supernatant and re-suspend the cell pellet in PBS or cell culture medium to adjust to a final concentration of 10-15 cells per field using the 40x objective.



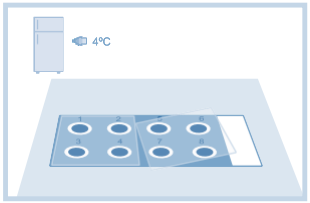
3.

Once the agarose (tube A) is at 37°C, first transfer 10 µl of CCs to a 0.2ml Eppendorf tube provided with the kit. Keep the tubes with the cells at 37ºC. Then add 20 µl of liquid agarose and mix well avoiding bubble formation. Keep the mix of cells and agarose at 37ºC until preparing the 8 samples to avoid premature gelification.



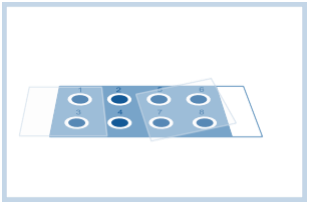
4.

Dispense a volume of 1.5-2 µl of the cell suspension into the marked wells and quickly cover every 4 wells with a 24x24 mm coverslip. Press gently avoiding the formation of air bubbles. Carryout the same action with the other 4 wells.
From this point on, the slide should be kept in a **horizontal position** throughout the process.



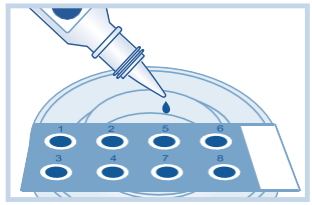
5.

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



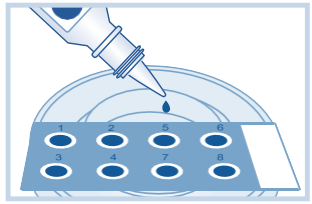
6.

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.



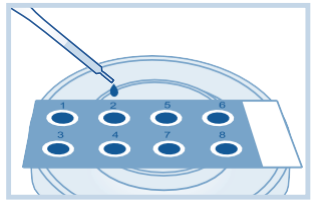
7.

Place the slide horizontally in an elevated position as suggested in the figure into a Petri dish or similar tray.
Apply Solution 1 (LS) on the well making sure **it is fully covered by the reagent during the whole process**. Incubate for 3 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 the slide.**



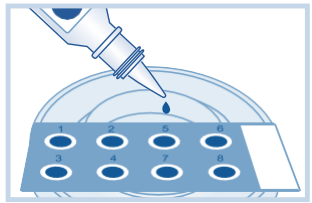
8.

Apply Solution 2 (SB) on the well making sure it is fully immersed. Incubate for 3 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 the slide.**



9.

Dehydrate the sample by applying 70% ethanol, using a disposable pipette and incubate for 2 minutes. Drain and apply 100% ethanol for 2 minutes. Drain and allow it to dry horizontally on filter paper or similar.

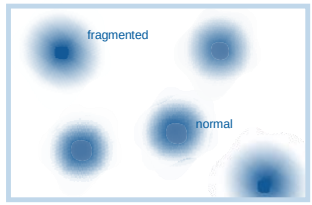


10.

Place the slide horizontally in an elevated position into a Petri dish or similar tray. Apply Solution 3 (SSA) on the wells ensuring they are fully immersed. Incubate for 15 minutes.Then, remove the stain by tilting the slide and place the slide horizontally in an elevated position.

11.

Apply Solution 4 (SSB) on the wells, again ensuring they are fully immersed. Incubate for 15 minutes. Then, remove the stain by tilting the slide. Briefly wash the slide with tap water. Remove the excess of water and allow it to dry at room temperature.



12.

Visualize under bright field microscopy. If the staining is too intense, the slide might be washed with tap water.

If the staining appears too weak, immerse the slide in 100% ethanol, allow it to dry and repeat the process from step 10.

For fluorescence microscopy staining, please contact the authorized dealer.

$$DFI\ (\%) = \frac{\text{Fragmented}}{\text{total number of counted cells}} \times 100$$

13.

Calculate the percentage of CCs with fragmented DNA. Given that the number of cells is limited and depends on how the CCs were obtained after denudation of the oocyte, the recommended option is to count all the cells observed on the slide. It is advisable to count a minimum of 100 cells.

For future assays

Use as many Eppendorf tubes as CCs samples are going to be evaluated. Place the Eppendorf tube (ET) into the float and melt using a water bath (or a beaker with water on a hot plate) at 95-100ºC for 5 minutes or until it is completely melted. Alternatively, if you prefer melting the agarose using a microwave oven, fill a beaker with 100 ml of water. Then, place the ET in the float inside the beaker and heat it at maximum power for 1.5 minutes. Monitor it constantly and stop the process as soon as the water starts boiling. Continue the protocol from point 3.

Please do not keep the ET boiling inside the microwave!