




oxiSperm® II

Kit **REF** HT-OS20II
for 20 determinations

oxiSperm® II is a simple test that allows the assessment of pro-oxidant activity in semen samples.

IVD for professional use only.

	consult instructions of use
	product reference (catalogue number)
	batch number
	use-by date
	manufacturer
	date of manufacture
	in vitro diagnostic medical device
	contains sufficient for "n" test
	temperature limit
	keep dry
	warning
	Keep away from direct sunlight



Principle of the method

oxiSperm® II consists on a established assay providing the professionals with a reliable technology for the assessment of pro-oxidant activity in neat semen, seminal plasma, and spermatozoa. The test is based on the Nitro Blue Tetrazolium assay (NBT) in the form of a specifically formulated pre-embedded reactive membrane. This assay is established on the reaction of pro-oxidant substances with the water-soluble tetrazolium salt to form insoluble crystals of formazan (Baehner et al, 1976). This molecule produces an increasing colour intensity in the membrane that ranges from a pale pink to a dark purple that can be easily and comparatively classified by visual inspection (Fig. 1). This test is an aid in the diagnosis. Interpretation of the results will be under medical criteria.

Performance characteristics

Sensitivity	98.64 %
Specificity	99.01 %
Repeatability	98.09 %
Reproducibility	97.12 %
Trueness	98.32 %
Accuracy	96.38 %
Interferent substances	-

Description of kit reagents

Every kit contains the necessary to perform the assay in 20 ejaculates. The components are:

- (RS) Reactive membranes; 20 units
- (SI) Induction solution; 210 µl
- Instructions for Use

Material and equipment required not provided with the kit

Micropipettes, centrifuge capable of reaching 6000xg, Eppendorf tubes, Phosphate buffer saline (PBS), PVC (polyvinyl chloride) gloves.

Beware that all equipment is calibrated.

Sperm sample

Fresh semen samples are advisable and should be collected in a sterile recipient. Frozen semen samples can be used for the analysis of pro-oxidant activity in neat semen or seminal plasma but not in spermatozoa.

IFU

Use recent ejaculated semen samples to obtain the most realistic information at the time of ejaculation.

1. From the semen sample received, put 10 µl aside in a new Eppendorf tube. This is the **Neat Semen Sample (N)** that will be used in step 7.
2. Then, put aside a volume with at least 106 spermatozoa in a new Eppendorf tube. Centrifuge at 6000xg for 10 minutes.
3. After centrifugation, transfer the supernatant to a new Eppendorf tube. This is the **Seminal Plasma Sample (SP)** that will be used in step 7.
4. Once supernatant is set aside, re-suspend the spermatozoa pellet in 50 µl of PBS and incubate it for 3 minutes at room temperature.
5. Centrifuge at 6000xg for 10 minutes. Discard supernatant and re-suspend spermatozoa pellet in 5-10 µl of the Induction solution (SI). Incubate for 5 minutes. This is the **Sperm Sample (S)** that will be used in step 7.
6. Pull out from the envelope the reactive membrane card containing 4 wells, 1 for each sample and a control. Avoid direct sunlight exposure on the membrane.
7. Place 5 µl of the content of each Eppendorf tube (**Neat semen sample, Seminal plasma sample, Sperm sample**) at the corresponding well on the reactive membrane (**N, SP, S**, respectively), and 5 µl of PBS on the control (**C**) well.
8. Let the membrane at room temperature protected from direct sunlight exposure. Full colour will develop in 15 minutes.
9. Compare the colour of each well with the colour palette in Fig. 1.

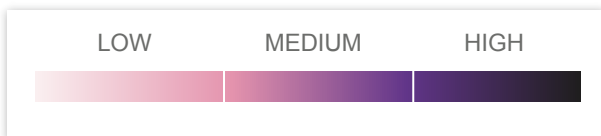


Figure 1. Colour pallet according to NBT- reactivity (according to oxiSperm®II).

Warnings, environment and precautions

All patient samples and reagents should be treated as potentially infectious, and users must wear protective gloves, eye protection and laboratory coats when performing the test.

Ensure good ventilation of the workstation. Avoid contact with skin and eyes. Wear personal protective equipment.

Do not touch the membrane directly with the hands or skin. This can modify the expected reaction. Avoid direct exposure of the reactive membrane to sunlight. Keep the unused reactive membranes in the wrapper protected from light.

Do not eat, drink or smoking in the area where samples and assay reagents are handled.

Do not use beyond the expiration date, which appears on the package label.

Once the test is performed, do not re-store the card to reuse those wells that have not been used. The reactive support of unused wells may be affected by exposure to artificial light during incubation for colour development in the wells in which the sample has been applied.

Do not release the products used into the environment. Follow centre guidelines for the storage and disposal of toxic substances. Membranes and all material used to perform the test should be discarded to a proper biohazard container after testing.

Store conditions

After receiving the kit, store it at 2-27 °C and keep it protected from light. After opening the kit, it is recommended to store it at 2-8 °C.

Expiry date is on label. After opening, the kit is stable for 12 months.

