halotech®

oxiSperm® II

Kit REF HT-OS20II
for 20 determinations

consult intructions of use
REF product reference
LOT lot number
use by
manufacturer
IVD in vitro diagnostic
contains sufficient for "n" test
2°C 27°C temperature limitation
keep dry
keep away from direct sunlight

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

Principle of the method

oxiSperm® II has been developed for the assessment of pro-oxidant activity. However, these test are not habitually used by clinical and andrology laboratories. oxiSperm® II provides the clinicians with an easy, fast, reliable and stablished assay for the determination of pro-oxidant activity at three levels: neat semen, seminal plasma or spermatozoa. The test is based in the Nitro Blue Tetrazolium assay (NBT) in the form of a specifically formulated pre-embedded reactive membrane. This assay is based on the reaction of pro-oxidant molecules with the water soluble tetrazolium salt to form water insoluble blue crystals known as formazan (Baehner et al, 1976). These crystals produce an increasing colour intensity in the membrane that ranges from a pale pink to a dark purple-blue that can be easily and comparatively quantified by visual inspection (see colour palette).

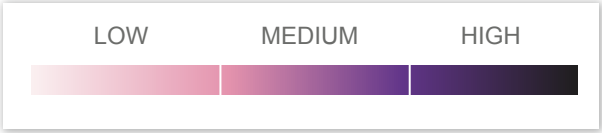

Figure 1. Colour pallet according to NBT- reactivity (according to oxiSperm®II)

Description of kit reagents

Each kit contains reagents to test the pro-oxidant activity in 20 ejaculates (neat semen, seminal plasma, sperm).

OSIIRS: Reactive membranes. 20 units with 4 wells each
(N: Neat Semen; SP: Seminal Plasma; S: Sperm; C: Control).
OSIISI: Sperm reactivity induction solution. 1 vial with 210µl.

Material and equipment required, not provided with the kit

*Micropipettes
*Centrifuge capable of reaching 6000xg
Eppendorf tubes
Phosphate Buffered Saline (PBS)
*** NOTE: All equipment should be calibrated**

Samples

Fresh semen samples are advisable. Frozen semen samples can be used for the analysis of pro-oxidant activity in net semen or seminal plasma but not in spermatozoa

Assay protocol

Use recent ejaculated semen samples to obtain the most realistic information at the time of ejaculation. Frozen semen samples can be used for the analysis of oxidative stress in net semen or seminal plasma, but not in spermatozoa.

1. Add 10 µl of neat semen sample to a clean Eppendorf tube -Neat Semen Sample-.
2. Transfer an additional volume of net semen containing at least 10^6 spermatozoa to a new Eppendorf tube or similar, and centrifuge the sample at 6000xg for 10 minutes Transfer seminal plasma fraction to a clean Eppendorf tube -Seminal plasma sample-.
3. Re-suspend spermatozoa pellet in 50 µl of phosphate buffered saline (PBS) and keep it for 3 min at room temperature.
4. Centrifuge spermatozoa at 6000xg for 10 minutes. Discard supernatant and re-suspend spermatozoa pellet in 50µl of PBS and repeat centrifugation at 6000xg for 10 minutes. Discard supernatant and re-suspend spermatozoa pellet in 5-10 µl of the sperm reactivity induction solution. Keep the sperm in these conditions at room temperature for 5 min -Sperm sample-.
5. Pull out from the envelope the reactive membrane card containing 4 wells, 1 for each sample and control. Avoid direct sunlight exposure on the membrane.
6. Place 5 µl of the content of each Eppendorf tube (Neat seminal sample, Seminal plasma sample, Sperm sample) at the corresponding place on the reactive membrane.
7. Let the membrane at room temperature protected from direct sunlight exposure. Full colour will develop in 15 minutes (colour development in spermatozoa may take a prolonged time).
8. For a negative control, the control well can be used by adding 5 µl of PBS.
9. Compare the colour of the samples with the colour of the scheme (Figure 1).

Precautions

1. All samples should be treated as potentially infectious and users must wear protective gloves, eye protection and laboratory coats when performing the test.
2. 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.
3. Membranes and all material used to perform the test should be discarded to a proper biohazard container after testing.
4. Do not eat, drink or smoking in the area where samples and assay reagents are handled.
5. Do not use beyond the expiration date, which appears on the package label.
6. Material Safety Data is available upon request.
7. 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 color development in the wells in which the sample has been applied

Safety and the environment

Do not release the products used into the environment. Follow center guidelines for the storage and disposal of toxic substances.
Biological samples must be handled as potentially infectious.

Storage conditions

After receiving the kit, store it between 2°C and 27°C and keep it protected from light. After opening the kit, it is recommended to store it at 4ºC.

Expiration

The reagents supplied are stable for 12 months.

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