Points for consideration:

- Perform the test on the freshly collected semen sample.
- Process the sample immediately after liquification.
- This test is mend only for fresh ejaculated undiluted semen sample.
- Addition of exact semen volume as mentioned in procedure is mandatory to get accurate result.
- After melting the gel at 90°C -100°C, perform rest of the steps at 37°C. Maintaining temperature is mandatory.
- The temperature for mixing of the gel and semen sample must be kept 37°C, as high temperature will increase ROS and low temperature will allow the gel to get solidify.
- Record the result exactly after 55 minutes otherwise the intensity of the colour may change and can lead to false positive results.

Precautions:

1. All samples and reagents must be treated as potentially hazardous.
2. The performer must wear disposable gloves, eye protection and laboratory gowns when performing the process.
3. The solutions and reagents must be discarded in a proper biohazard container at the end of process.
4. The kit reagents beyond the labeled expiration date should not be used for sample processing.
5. One should not eat, drink, or smoke in area where the sample and kit reagents are handled.

Troubleshooting:

If there is some abnormal colour indication then repeat whole test.

CANDORE

CE

CANros

REF : CA-002

(Kit to determine Reactive Oxygen Species in human semen)

Technical Assistance:

CANDORE Bioscience
1st floor, Shashwat Complex, Naranpura, Ahmedabad, Gujarat, India 380013.
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Irritant | Toxic | Flammable | Use by | LOT Lot number | REF Product reference | Manufacturer | Consult instruction of use

Introduction:

This test is based on reduction of Nitro blue tetrazolium dye to determine the total ROS generated in human semen by leucocytes and spermatozoa. When REACTIVE AGENT interacts with the free radicals present in the semen sample, it generates colour varying from light pink to dark purple. The colour change depends upon the concentration of the free radicals present in the sample.

Materials and Reagents Provided:

	3 Test (SAMPLE)	10 Test	20 Test
▣ Agarose N-Gel tubes	03 No.	10 No.	20 No.
▣ Float	01 No.	01 No.	01 No.

Reagents and Equipment required but not provided:

- ▣ Disposable Gloves and mask
- ▣ Dropper
- ▣ Micropipettes
- ▣ Micropipette Tips
- ▣ Water bath
- ▣ Dry bath / Incubator/ Heat plate

Storage and Stability:

- ▣ The kit must be stored at 4-25°C temperature.
- ▣ The kit reagents beyond the labeled expiration date should not be used for sample processing.

Procedure:

- ▣ Collect the sample in the sterile semen collection jar. Allow the sample to get liquefy at 37ºC, avoid manual liquification if possible.

  Note: If the sample is too viscous, then liquefy with the help of dropper avoiding bubble formation. For better results perform the result within 30 minutes of sample collection.
- ▣ Place the provided Agarose N-Gel tube in float and incubate in boiling water at 90°C -100°C for 2 minutes or until gel melts.
- ▣ Place the melted gel tube at 37°C for 5 minutes.
- ▣ Add 200µl of semen sample to the melted Agarose N-Gel tube and mix gently by avoiding bubble formation and stress to sperm cells.
- ▣ Incubate the tube for 55 minutes at 37°C.
- ▣ After incubating for 55 minutes, observe the colour change immediately and compare with the colour code as mentioned below to determine the level of the oxidative stress present in the sample.

Interpretation of the result:

Interpretation	Colour	Colour Code
Normal	White / Light pink	
Low level of ROS	Light purple	
Moderate level of ROS	Purple	
High level of ROS	Dark purple	