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Instructions for Use

ORIGIO® Gradient™ 100

Product No.:
8400

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a CooperSurgical Company

ORIGIO® Gradient™ 100

For the separation of motile sperm from the ejaculate by the density gradient method.

Pack size

84000060D ORIGIO® Gradient™ 100 (60 ml)
84002060D ORIGIO® Gradient™ 100 (2x60 ml)
84000125D ORIGIO® Gradient™ 100 (125 ml)
84004125D ORIGIO® Gradient™ 100 (4x125 ml)

Composition

Silane-coated silica particles
Glucose
Sodium pyruvate
Physiological salts
Taurine
HEPES
Sodium bicarbonate

Quality control testing

Sterility tested (Ph.Eur., USP)
Osmolality tested 297-313 mOsm/kg (Ph. Eur., USP)
pH tested 8.0-8.5 (Ph.Eur., USP)
Endotoxin tested ≤0.8 EU/ml (Ph.Eur., USP)
Sperm survival tested motility ≥80% of control
Note: The results of each batch are stated on a Certificate of Analysis, which is available on www.origio.com.

Buffer system

ORIGIO® Gradient™ 100 is a fully HEPES buffered medium and does not require pre-incubation before use

Storage instructions and stability

Store in original container at 2-8°C, protected from light.
Do not freeze.
Discard excess (unused) media following warming.
The product is to be used within 28 days after opening.
When stored as directed by the manufacturer the product is stable until the expiry date shown on the label.

Precautions and warnings

Do not use the product if:

1. Product packaging appears damaged or if the seal is broken.
2. Expiry date has been exceeded.

Caution: U.S. federal law restricts this device to sale by or on the order of a physician (Rx only).

Note: Please be aware that precipitation (colloidal aggregates) may occur. Decanting the medium is recommended when precipitation is present

Instructions for use

1. For custom gradient preparation use below chart to prepare a chosen gradient, using ORIGIO® Gradient™ 100 and ORIGIO® Sperm Wash

ORIGIO® Gradient™ 100	ORIGIO® Sperm Wash	End result
8 ml	2 ml	**= 10 ml lower phase 80 % solution**
4 ml	6 ml	**= 10 ml upper phase 40% solution**

2. Bring all components to room temperature before use.
3. For each 1-2 ml of semen sample used, prepare a separate gradient using 1-2 ml of upper phase (40 % ORIGIO® Gradient™) carefully underlain with 1-2 ml of lower phase (80 % ORIGIO® Gradient™.)
   Optimal results may be achieved if gradients are prepared shortly before use.
4. Carefully dispense 1-2 ml of liquefied semen sample on top of the prepared gradient.
   Care should be taken not to overload the gradient as this may result in a poor sperm yield.
5. The gradient is centrifuged at 300-400 g for 15-20 minutes.
   Centrifugation speed and duration may be modified to improve the quality and/or yield of sperm collected, depending on the initial semen characteristics.

6. Remove the supernatant from the pellet and transfer the pellet with a new sterile tip into a clean conical centrifugal test tube containing 3-5 ml of ORIGIO® Sperm Wash.
7. Centrifuge at 200-300 g for 5-10 minuts. Aspirate and remove most of the supernatant.
   Repeat this washing procedure.
8. Finally re-suspend the pellet in appropriate medium according to further procedure; determine the motility and concentration of spermatozoa.
9. If needed, further dilute the re-suspended sperm pellet in order to obtain the required sperm concentration.

Note: For IVF procedures it is advised to use a specialized fertilization medium (e.g. ORIGIO® Sequential Fert™) when re-suspending the pellet (step 8/9).
For ICSI procedures where no immobilization medium (e.g. PVP, SpermSlow™) is used for sperm selection, it is advised to use an alternative holding medium when re-suspending the pellet (step 8/9).

Note: Prolonged storage of re-suspended sperm pellets before use, no matter the media used, may increase the sperm DNA fragmentation index, especially in male factor infertility cases. [1, 2]

[1] Jackson R.E, et al., Effects of semen storage and separation techniques on sperm DNA fragmentation. Fertil Steril. 2010;94(7):2626–2630.

[2] Balasuria A, et al., Processes involved in assisted reproduction technologies significantly increase sperm DNA fragmentation and phosphatidylserine translocation. Andrologia. 2014 Mar; 46(2):86-97