FertiCult™ G3 medium

Cell culture medium for in vitro culture of human embryos, from 48 hours till 96 hours in culture

STERILE A

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GENERAL INFORMATION

FertiCult G3 medium is a ready to use medium for the *in vitro* culture of mammalian embryos. It is designed for sequential culture with FertiCult IVF medium from 48 hours till 96 hours culture (day 3 and 4).

The medium is complete and does not require further additives. If preferred patient serum can be added.

FertiCult G3 medium is suitable for micro-droplets under oil or equally suited to culture in Falcon 3037 organ culture dishes (also 4-well Nunc culture dishes).

As with all IVF media, G3 medium has to be **pre-incubated in CO_2 incubator for 24 hours before use** (with lid opened).

MATERIAL INCLUDED WITH THE KIT

Product code	Product description
G3020	5x 20ml FertiCult G3 medium
G3050	5x 50ml FertiCult G3 medium

MATERIAL NOT INCLUDED WITH THE KIT

- Incubator at 37°C (5% CO_2)
- Petri dishes (e.g. Falcon 3037)
- Mineral oil (e.g. FertiCult Mineral Oil)
- Laminar flow bench (ISO5 environment)
- Microscope
- Test tubes

PRODUCT SPECIFICATIONS

- Chemical composition
- pH: 7.30 - 7.60 (37°C - 5% CO_2)
- Osmolality: 270 - 290 mOsm/kg
- Sterility: sterile (SAL 10^{-3})
- Endotoxins: < 0.25 EU/ml
- Mouse Embryo Assay (blastocysts after 96h culture): ≥ 80% (from zygote stage)
- Use of Ph Eur or USP grade products if applicable
- Certificate of analysis and MSDS are available upon request

PRE-USE CHECKS

- Do not use the product if it becomes discoloured (if medium contains phenol red), cloudy, or shows any evidence of microbial contamination
- Do not use the product if seal of the container is opened or defect when the product is delivered

STORAGE AND CONSERVATION

- Store between 2-25°C, once opened store between 2-8°C
- Do not freeze before use
- Keep away from (sun)light
- After opening the container do not use the product longer than 7 days
- Do not use after expiry date

WARNINGS AND PRECAUTION

FertiCult G3 medium contains human serum albumin. Source materials from which this product was derived was found negative when tested for antibodies to HIV and HCV and non-reactive for HbsAg, HBV RNA, HCV RNA, HIV-1 RNA and syphilis. No known test method can offer assurance that products derived from human blood will not transmit infectious agents. Therefore, handle all specimens as if capable of transmitting HIV or hepatitis.

Always wear protective clothing when handling specimens.

Always work under strict hygienic conditions (ISO 5 environment, e.g. LAF-bench) to avoid possible contamination, even when FertiCult G3 medium contains antibiotics.

FertiCult G3 medium does not contain antibiotics, always work under strict hygienic conditions (laminar flow) to avoid contamination, or add your own antibiotics: penicillin at about 100 units per ml or gentamicin at 10mg per liter.

METHOD

PREPARATIONS FOR USE OF MICRO-DROPLETS

For micro-droplets, between 100-250µl of FertiCult G3 medium may be dispensed around the culture dish, up to 6 per 60mm dish.

The dish is then filled with 5ml of pre-washed and pre-equilibrated light mineral oil (nontoxic and preferably embryo tested, e.g. FertiCult Mineral Oil).

Each dish is placed in the incubator (usually non-humidified) to equilibrate overnight at 37°C and under an atmosphere of 5% CO_2 in air.

PREPARATIONS FOR USE IN OPEN SYSTEMS

In open systems such as with the Falcon or Nunc dishes, about 1ml of medium is placed in each well.

A further 3ml is placed in the reservoir surrounding the wells. This helps to maintain humidity as well as providing medium with which to wash the embryos.

Each dish is placed in the incubator to equilibrate overnight at 37°C and under an atmosphere of 5% CO_2 in air.

As with the micro-droplets method described above, **equilibration overnight is highly recommended**.

In the open system, paraffin oil is not necessary, but sometimes a 1ml layer of mineral oil may be added over the medium in the inner well. When using such an open system the incubator must be humidified.

CULTURE

After 48 hours culture, embryos are usually ready for embryo transfer. However, if further culture is intended, use FertiCult G3 medium.

Place the embryos into fresh dishes containing fresh FertiCult G3 medium (see above).

It is important to prepare fresh dishes for this purpose and they must be equilibrated by overnight pre-incubation and pre-equilibration in the incubator. After a further 24 hours culture the embryos will then be ready for transfer (between 8 and 16 cells at this stage).

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