SAGE Media™

# *In Vitro* Maturation Media Kit

**For laboratory procedures only; other uses must be qualified by the end user.**

| Product Description | REF Number | Kit Size |
|---|---|---|
| Oocyte Washing Medium | ART-1600-A | Sufficient for one (1) oocyte culture procedure for preparation for ART |
| Oocyte Maturation Medium | ART-1600-B | |
| Embryo Maintenance Medium | ART-1600-C | |

## INTENDED USE

These products are intended for the preparation of oocytes for use in A.R.T. Procedures.

## PRODUCT DESCRIPTION

The components of this kit will allow for the preparation of collected immature oocytes, their culture to prepare them for fertilization, and the subsequent culture of embryos derived from them.

This product contains 10 mg/L of gentamicin, an aminoglycoside antibiotic.

## MATERIALS PROVIDED IN THE IN VITRO MATURATION MEDIA KIT

1 x 50 mL vial of Oocyte Washing Medium (REF # ART-1600-A)

1 x 20 mL vial of Oocyte Maturation Medium (REF # ART-1600-B)

1 x 5 mL vial of Embryo Maintenance Medium (REF # ART-1600-C)

## PRECAUTIONS AND WARNINGS

a. The clinical pregnancy and birth rates are lower for IVF Cycles using oocytes matured *in vitro* compared to conventional IVF cycles.

The clinibal benefit of *in vitro* maturation in IVF cycles is limited to the ability to harvest (immature) oocytes with little or no hormonal stimulation to the ovary.

c. Relatively fewer mature oocytes are available for fertilization following *in vitro* maturation compared to the number of oocytes obtained by aspiration following conventional ovarian stimulation protocols.

d. The *in vitro* oocyte maturation involves a significantly lower risk of ovarian hyperstimulation syndrome compared to conventional IVF.

Do not use medium that shows evidence of particulate matter or cloudiness, or is not rose colored.

The Oocyte Washing Medium (ART-1600-A) component in this kit contains 5 mg/mL Serum Protein Substitute.

The Oocyte Maturation Medium (ART-1600-B) component in this kit contains 5 mg/mL Serum Protein Substitute.

The Embyro Maintenance Medium (ART-1600-C) component in this kit contains 5 mg/mL Serum Protein Substitute.

**Caution:** All blood products should be treated as potentially infectious. Source material from which this product was derived was found negative when testing for antibodies to HIV-1/HIV-2 and HCV and non-reactive for HBsAg, HCV RNA and HIV-1 RNA. No known test methods can offer assurances that products derived from human blood will not transmit infectious agents. Donors of the source material have been screened for Creutzfeldt-Jacobs disease (CJD). Based on effective donor screening and product manufacturing processes, it carries an extremely remote risk for transmission of viral diseases. A theoretical risk for transmission of CJD is also considered extremely remote. No cases of transmission of viral diseases or CJD have ever been identified for albumin.

Standard measures to prevent infections resulting from the use of medicinal products prepared from human blood or plasma include selection of donors, screening of individual donations and plasma pools for specific markers of infection and the inclusion of effective manufacturing steps for the inactivation/removal of viruses. Despite this, when medicinal products prepared from human blood or plasma are administered, the possibility of transmitting infective agents cannot be totally excluded. This also applies to unknown or emerging viruses and other pathogens. There are no reports of proven virus transmissions with albumin manufactured to European Pharmacopoeia specifications by established processes.

**Single use:** To avoid problems with contamination, handle using aseptic techniques and discard any excess product that remains in the bottle or vial after procedure is completed.

Reproductive media products are intended for single use only. Re-use of reproductive media may result in using a product past its labeled expiration date or increase the risk of microbial contamination in a subsequent procedure if the practitioner fails to utilize adequate aseptic techniques. Use of expired or microbial contaminated product may result in suboptimal conditions to promote fertilization and/or embryo quality during in-vitro culture. These conditions may result in the failure of the embryo to develop properly or to implant, potentially leading to a failed assisted reproductive procedure.

**Note:** Embryo is considered a general term. More precisely, SAGE™ considers the period of time initiating when a single diploid cell results from the fusion of male and female genome resulting in zygote formation with subsequent development from repeated mitotic divisions forming a solid mass or morula (typically day 4-5) and after which a fluid-filled cavity develops resulting in blastocyst formation (typically day 5-6) ending with embryo implantation that begins the end of the first week and is completed by the end of the second week post conception.

**Caution:** U.S. Federal law restricts this device to sale by or on the order of a physician (or properly licensed practitioner).

This product contains the antibiotic gentamicin sulfate. Appropriate precautions should be taken to ensure that the patient is not sensitized to this antibiotic.

## QUALITY ASSURANCE

One-cell MEA tested and passed with 80% or greater blastocyst. USP Endotoxin tested and passed with <1 EU/mL.

A Certificate of Analysis is available for this product.

## DIRECTIONS FOR USE

1. Cumulus-oocyte-complexes (COCs) are collected by aspiration into culture tubes containing approximately 2-3 mL of heparinized (2 IU/mL of heparin) warm Quinn's Advantage™ Medium with HEPES (Ref # ART-1023/1024); this product is NOT supplied in the kit.
2. Preparation of OOCYTE WASHING MEDIUM prior to oocyte retrieval: OOCYTE WASHING MEDIUM must be prepared at least one hour before oocyte collection and kept at 37 °C. Briefly, three 35 x 10 mm Petri dishes, each containing approximately 2.0-2.5 mL of OOCYTE WASHING MEDIUM under Oil for Tissue Culture (REF # ART-4008), are prepared for every patient. If a cell strainer (Falcon, Cell Strainer 352350, 70 µm Nylon; www.bd.com/labware) is to be used to collect COCs, a 50 mL flask containing approximately 25-30 mL of OOCYTE WASHING MEDIUM must also be prepared for each patient and kept in an incubator at 37 °C; if kept in a 5% $CO_2$ incubator, make sure that the flask cap is tightened to avoid a deleterious shift in pH.
3. Preparation of OOCYTE MATURATION MEDIUM: COCs with no 1st polar body (1PB) are incubated in an Organ Tissue Culture Dish (Falcon, 60 x 15 mm) containing 1 mL OOCYTE MATURATION MEDIUM supplemented with a final concentration of 75 mIU/mL FSH and 75 mIU/mL LH at 37 °C in an incubator with an atmosphere of 5% $CO_2$ and 95% air with high humidity (or with triple gas mixture (90% $N_2$, 5% $CO_2$, and 5% $O_2$) and 100% humidity). A maximum of 10 COCs should be cultured in each dish. The OOCYTE MATURATION MEDIUM should be prepared for equilibration at least two hours before oocyte retrieval (practically, it can be made one day before).
   a. Place 10.0 mL of OOCYTE MATURATION MEDIUM into a test tube;
   b. Dissolve completely 1 ampoule of 75 IU FSH and 1 ampoule of 75 IU LH into (a).
   c. Place 9.90 mL of fresh OOCYTE MATURATION MEDIUM into a test tube.
   d. Take 100 µL FSH and LH dissolved as per item (a) and transfer into (c).
   e. Prepare three Organ Tissue Culture Dishes for each patient. In each dish, the inner well contains 1 mL of (d) and the outer well 2 mL of (d);
   f. Cover the Organ Culture Dish with the dish cover and place it in the incubator.

Detailed instructions concerning the source and addition of the FSH and LH to the OOCYTE MATURATION MEDIUM are given in the publication "Handbook on In Vitro Maturation of Immature Human Oocytes", R-C Chian, 2006, available upon request from SAGE™ Customer Service or your local SAGE™ sales representative. Sources of FSH and LH include: Repronex™ from Ferring Inc. (this is a combination of FSH and LH); Humegon™ from Organon (this is a combination of FSH and LH); Follistim™ from Organon (this is recombinant FSH); Gonal-F™ from Serono (this is recombinant FSH); and Pregnyl™ from Organon (this is hCG which has the same biological activity as LH). Other sources of pharmaceutical grade FSH, LH and hCG are also appropriate.

4. Sperm Preparation: Semen can be collected and prepared for insemination on the day of oocyte retrieval if an oocyte with 1PB has been retrieved. Otherwise, semen collection and preparation should be performed the day after oocyte retrieval. If possible, a fresh sperm sample should be obtained which can then be prepared for the insemination.
5. Stripping Oocytes 24 Hours After Culture: The COCs are cultured in OOCYTE MATURATION MEDIUM for 24 to 48 hours. Twenty-four hours after initiation of culture, all of the COCs are stripped for identification of oocyte status. COCs are denuded of cumulus cells using a fine bore pipette of appropriate diameter following one minute of exposure to a licenced product approved for this procedure NOT supplied in the kit. Oocytes with 1PB are then subjected to insemination by either IVF or ICSI after stripping. The remaining oocytes at germinal vesicle or metaphase I stage continue in culture for another 24 hours. At this point, it is not necessary to change the OOCYTE MATURATION MEDIUM.
6. Forty-eight hours after oocyte retrieval, the remaining stripped oocytes are re-examined and if any have 1PB, they are inseminated immediately by either IVF or ICSI. ICSI is recommended for the insemination of oocytes with 1PB because this method probably offers a greater chance of successful fertilization than does IVF.
7. Preparation of EMBRYO MAINTENANCE MEDIUM: EMBRYO MAINTENANCE MEDIUM must be prepared at least 2-4 hours before ICSI and kept at 37 °C in an incubator with an atmosphere of 5% $CO_2$ and 95% air with high humidity or with triple gas mixture (90% $N_2$, 5% $CO_2$, and 5% $O_2$) and 100% humidity. Briefly, prepare 20 µL droplets under mineral oil (Oil for Tissue Culture; Ref # ART-4008) in a 35 X 10 mm culture dish. The number of dishes used for each patient will depend upon the number of 1PB oocytes obtained after oocyte retrieval and culture.
8. After ICSI, the individual oocyte is transferred into a 20 µL droplet of EMBRYO MAINTENANCE MEDIUM in the culture dish and placed in the incubator for culture.
9. Identification of Fertilization: Sixteen to 18 hours after ICSI, fertilization of the oocytes is checked under a microscope for the appearance of two distinct pronuclei (2PN) and two polar bodies. At this point, it is not necessary to transfer the fertilized oocytes (2PN embryos) into another medium (dish) for continued culture.
10. Embryo Culture: The fertilized oocytes are to be cultured in the droplets (20 µL) of EMBRYO MAINTENANCE MEDIUM under Oil for Tissue Culture (REF # ART- 4008) for one or two additional days, depending upon the number and quality of embryos achieved. If culture to the blastocyst stage is desired, the cleaved embryos should be transferred into new droplets (20 µL) of EMBRYO MAINTENANCE MEDIUM in a culture dish under Oil for Tissue Culture (REF # ART-4008) three days after ICSI.

**Each laboratory should make its own determination of the particular details to use for each particular procedure.**

## STORAGE INSTRUCTIONS AND STABILITY

Store unopened containers refrigerated at 2 °C to 8 °C. Warm to incubator (37 °C) temperature prior to use. Do not freeze or expose to temperatures greater than 39 °C. The products are stable until the expiration date shown on the label.

1. Remove desired volume of product using aseptic procedures. One vial contains adequate volume of medium for one procedure.
2. Once removed, do not return any volume of product to the original container. Discard remaining product.
3. Do not use if the product becomes discolored, cloudy or turbid, or shows any evidence of microbial contamination.

## RELATED PRODUCTS

ART-4008P Oil for Tissue Culture

SAGE™ In Vitro Fertilization™ has a full line of products for the Reproductive Medicine Specialist. Please call or write for specific information or to receive a copy of our current catalog. For technical questions, or to reach our Customer Service Department, call the SAGE™ Support Line.

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Gonal-F™ is a registered trademark of Serono Laboratories.

**SAGE™ SUPPORT LINE:**
**In the U.S.: (800) 243-2974**
**International: (203) 601-9818**

## EXPLANATION OF SYMBOLS

| | |
|---|---|
| REF | Catalog Number |
| LOT | Batch Number |
| | Use By (year, month, day) |
| | Do Not Reuse |
| 2 °C 8 °C | Temperature Limitation |
| STERILE A | Aseptic Technique Sterilization Membrane Filtered (SAL $10^{-3}$) |
| | **ATTENTION:** See instructions for use. |
| | Manufacturer |
| Rx Only | U.S. Federal law restricts this device to sale by or on the order of a physician (or properly licensed practitioner). |

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5650 ver. 2 : 2022.May.25