RENOVA LIFE INC.

SAR IVM medium

Product Description

SAR IVM medium (Catalog#: **SAR IVM 01, 02**) is **Simple and Reliable (SAR)** medium for oocyte maturation *in vitro* (IVM). It is specifically designed for a **bovine *in vitro* fertilization (IVF)** process. A high blastocyst development rate is guaranteed. This medium is developed with Renova Life Inc. (RLI) standardized recipes that have been proven to be perfectly suitable for *in vitro* production of bovine embryos in several peer-reviewed publications. Years of our research and experience have been put into the development of this medium, offering you the best quality and performance for IVM/IVF/IVC. Specifically tested embryo-water and embryo-bovine serum albumin (BSA) are used in our RLI in-house SAR IVM/IVF/IVC products, which have been well tested for bovine IVF and embryo culture *in vitro* and for making IVF and OPU-IVF embryos for cryopreservation and embryo transfer practices.

RLI **SAR IVM medium** is a constant high quality product, ideal for oocyte maturation and production of embryos *in vitro*. It is prepared for commercial and research use. RLI **SAR IVM medium** has been prepackaged as follows: **SAR IVM 01**, 1 mL/vial, 5 vials; **SAR IVM 02**, 4 mL/vial, 5 vials.

SAR IVM contains Medium 199 (M199) with Earle's salts, L-glutamine, sodium bicarbonate, and HEPES, supplemented with 10% (v/v) fetal calf serum, 0.5 µg/mL ovine FSH, 5.0 µg/mL ovine LH and 1.0 µg/mL estradiol, antioxidant and other small molecules that promote oocyte maturation *in vitro*.

Storage temperature: **Store at -20 °C upon arrival**. **Expiration: 2 months after production date.**

Protocol for IVM

A. Making IVM dishes

1. Prepare dishes early morning. Mark the 35 mm Petri-dishes (Falcon 1008) with marker pen (IVM dish # and date).

2. Using pipetman p200 make 7 drops of **SAR IVM** with 75 µL per drop in each dish. Cover the drops with 3 ml mineral oil.

4. Prepare several 35 mm Petri-dishes with 3mL 10 % FBS M-199 per dish.

5. Place **SAR IVM** and 10% FBS M-199 dishes in CO_2 incubator. The dishes should be balanced in the incubator 2-4 h prior to use.

B. Aspirating ovaries and searching oocytes

1. Set the ovary aspiration stations by placing three paper towels on top of a square Aluminum foil. Place latex gloves, syringes and needles beside each station. Keep glass beakers (for holding the aspirated ovaries) and 50 mL conical tubes (for collecting the aspirated follicular fluid).

2. With gloved hand, scoop a handful of ovaries from the cooler and place them on the paper towel to mop excess water/saline. With an 18 g needle attached to a 10 mL syringe, aspirate all follicles with size from 2 to 8 mm in diameter from ovary surface.

3. After aspirating, remove the needle from syringe, and push the syringe to empty follicular fluid into a 50 mL conical tube.

RENOVA LIFE INC.

4. Settle down the oocytes and debris to the bottom of tube. Remove the supernatant and wash the pellet with RLI's **Oocyte Washing Prior to Maturation** (OWP). The pellet is washed 2-3 times with OWP and finally the contents are pulled into a square grid search dish. The oocytes are searched under stereo microscope and picked up from dish using the mouth tubing attached to fine pulled and polished glass pipette. The oocytes are collected in a 35 mm Petri-dish (Falcon 1008) containing 3 mL OWP/dish.

5. Search the dish thoroughly and pick up good quality cumulus-oocyte-complexes (COCs) (at least 4 layers of cumulus cells), COCs are washed in OWP twice and in M-199 twice. At various stages of washing, bad oocytes are discarded, so that the final M-199 dish contains a pool of good quality oocytes.

C. Maturation oocytes *in vitro*

1. Selected oocytes are put in groups of 25 each in each drop of maturation medium. Thus, each maturation dish (with 7 drops) contains 175 immature oocytes. Write the time, oocyte numbers and initials of the technician on the lid of each IVM dish.

2. Move COCs maturation dishes and kept in the CO_2 incubator at 5% CO_2, 39 ^{o}C and under conditions of maximum humidity. The oocytes are kept in this condition for 22 to 24 hours to complete *in vitro* maturation process ready for IVF.

IVM calculation

SAR IVM 01 contains 1 mL/vial maturation medium with 5 vials per kit, total 5 mL. **SAR IVM 02** contains 4 mL/vial maturation medium with 5 vials per kit, total 20 mL.

For each IVM trail, two Petri dishes are prepared with 7 drops per dish for IVF. If 25 oocytes are added into each fertilized drop, a total of 175 oocytes (25 × 7 = 175) can be matured. As a result, one package of **SAR IVM 01** (5 mL) can be used to mature about 1600 COCs (5000/75 x25 =1666), while **SAR IVM 02** (20 mL) about 6400 COCs.

References

1. Senatore E, Xu J, Novoa M, Gong G, Lin T, Bella A, Moreno JF, Mannino M, Tian CX, Presicce GA, Wu S, and Du F* Improved in vitro development of OPU-derived bovine (Bos taurus) embryos by group culture with agarose-embedded helper embryos. ***Theriogenology***; 2010; 74(9):1643-1651.

2. Xu J, Guo Z, Su L, Nedambale TL, Zhang J, Schenk J, Moreno JF, Dinnyes A, Ji W, Tian XC, Yang X and Du F* Developmental potential of vitrified Holstein cattle embryos fertilized in vitro with sex-sorted sperm. ***Journal of Dairy Science*** 2006; 89:2510–2518.

3. Sung LY, Du F*, Xu J, Chang W, Nedambale TL, Jiang S, Tian XC, and Yang X The differential requirement of albumin and sodium citrate on the development of in vitro produced bovine embryos. ***Reproduction Nutrition and Development*** 2004; 44:551-564.

Ordering Information

Cat # SAR IVM 01 **1 mL/vial, 5 vials, $80.00 plus S&H**

Cat # SAR IVM 02 **4 mL/vial, 5 vials, $250.00 plus S&H**

Other packaging and bulk ordering is available upon request.