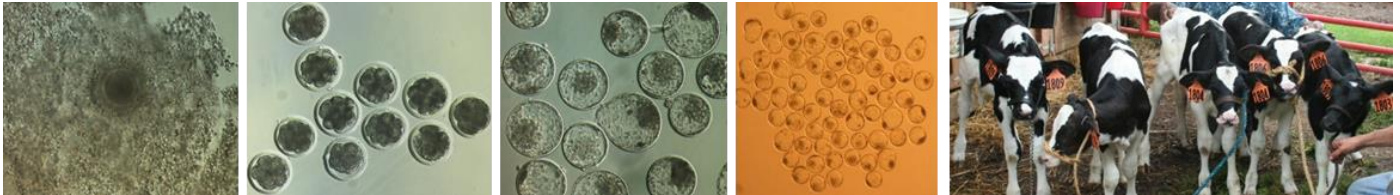


Complete Kit for OPU-IVF Embryo Production (CK-OPU-IVF 01)

Renova Life, Inc.

Ovum pickup, *in vitro* fertilization (OPU-IVF) and embryo transfer (ET) currently is a matured technology for cattle breeding and herd replacement program, and it is becoming a profitable business in America and Worldwide.



Cattle Breeding Program by *in vitro* Fertilization and Embryo Transfer

1. Complete Kit for OPU-IVF Embryo Production: CK-OPU-IVF 01

Renova Life, Inc. (RLI) has developed a whole package of Complete Kit for OPU-IVF Embryo Production: CK-OPU-IVF 01. CK-OPU-IVF 01 includes two kits (1) Complete Medium Kit for OPU-IVF: CMK-OPU-IVF 01, and (2) Complete Consumable Kit for OPU-IVF: CCK-OPU-IVF 01/02. RLI's CMK-OPU-IVF 01 includes high quality products with embryo culture tested, while CCK-OPU-IVF 01/02 contains sterile and disposable consumables to minimize the chance of contamination during IVF.

The complete kit of CK-OPU-IVF 01 can assist the veterinaries for OPU-IVF practice to make IVF embryos, in addition to MOET program to produce *in vivo* embryos by superovulation of donors, AI and embryo flushing. The veterinary who has worked on MOET program usually has a workable embryo laboratory for embryo flushing, searching and embryo cryopreservation by slow freezing procedures.

CK-OPU-IVF 01 kit is ready for use, and easy to handle.

The basic cost to produce one transferable blastocyst is affordable, at the range of \$5.54-\$11.09, dependent upon the number of donors assigned for each OPU-IVF trail.

2. Complete Medium Kit: CMK-OPU-IVF 01 is the medium kit specifically designed for OPU and IVF process, including oocyte retrieval, IVM/IVF/IVC and embryo transfer (ET) of fresh IVF blastocysts. The media are OAP (OPU oocyte aspiration and collecting), OWP (oocyte washing), SAR IVM (oocyte maturation), SAR IVF (oocyte IVF), SAR IVC-W (cumulus cell removal and oocyte washing post IVF), SAR IVC (embryo culture *in vitro*) and EWH (embryo washing and loading in ET straws prior to ET) (Table 1).



Complete Medium Kit for OPU-IVF: CMK-OPU-IVF 01

Table 1. Complete Medium Kit for OPU-IVF: CMK-OPU-IVF 01

Item name	Catalog #	Product Description	Unit Price (\$)	Quantity	Kit Price (\$)
OAP	RLI OAP 01	Oocyte Aspiration Plus, 500 mL	15	4	60
OWP	RLI OWP 02	Oocyte Washing Prior to IVM, 100 mL	18	4	72
Maturation	SAR IVM 01	SAR IVM medium, 1 mL/vial, 5 vials, 5 mL	80	1	80
IVF	SAR IVF 01	SAR IVF medium 100 mL	125	1	125
Oocyte Washing after IVF	SAR IVC-W 01	SAR IVF washing medium, 100 mL	55	2	110
Embryo Culture	SAR IVC 01	SAR IVC medium, 100 mL	150	1	150
Embryo holding for ET	EWH 02	Embryo Washing & Holding, 100 mL	25	1	25

Cat # CMK-OPU-IVF 01: \$622/kit plus S&H

3. Complete Consumable Kit: CCK-OPU-IVF 01 includes all consumable material and plastic dishes that can be used for whole process of OPU-IVF. The items are sterile, and shall be used as soon as opened in order to reduce the possibility of contamination (Table 2).



Complete Consumable Kit for OPU-IVF: CCK-OPU-IVF 01

Especially, RLI has developed an apparatus tool called Cumulus Remover (RLI CR 01), which is particularly designed for *an effective removal of cumulus cells from oocytes after IVF*. Cumulus removal from zygotes is critical for successful embryo culture and subsequently blastocyst development during OPU-IVF production.

Table 2. Complete Consumable Kit for OPU-IVF: CCK-OPU-IVF 01

Item name	Item Description	Unit Price (\$)	Quantity	Kit Price (\$)
Square Search Dish	10 cm X 10 cm plate for search oocytes, 10 dishes/pk	8	4	32
Petri-dishes	60 mm plastic dish for washing oocytes and embryos	6	4	24
Petri-dishes	35 mm plastic dish for making IVM/IVF/IVC dishes, and washing oocytes and embryos	7	12	84
4-well Dish	4-well dish for washing embryos before ET or freezing, 4 dishes/pk	7	2	14
Tip	200 µL, box	6	1	6
Tip	1000 µL, box	10	1	10
Disposable Transfer Pipette	Sterile and individual packed, disposable transfer pipettes, at the size of 2-3 mL, are used to remove supernatant after sperm washing and centrifuge during IVF process.	0.5	16	8
Cumulus remover	Cumulus remover is specially designed by RLI embryologist, for effective removal of cumulus cells from oocytes after IVF. Cumulus removal from zygotes is critical for successful embryo culture and blastocyst development during OPU-IVF production. Buy one set of Cumulus Remover; it can be used for several years. Recommended to Buy.	390	1	390
Cumulus removal glass set	Glass pipets made with the precise inner and outer diameter specific for removal cumulus cells after IVF, 15 needles/set. Refillable. Can be used for 15 IVF trials, each needle is disposable after use. Recommended to Buy.	150	1	150
ET Straws	Yellow 0.25 cc IMV sterile for direct transfer 5 straws per bag, 4 bags (20 straws) per pack. Optional. Each OPU-IVF session is expected to generate 25 blastocysts for embryo transfer (say 5 embryos/donor, 5 donors). For 4 times of OPU-IVF, 4 packs of ET straws are needed.	30	4	120
Mouth Piece Set	Mouth piece is used for mouth pipette to transfer the embryos from one dish to another. Optional.	35	1	35
Disposable Glass Pipettes	For dish-to-dish embryo and/or oocyte transfer. 5 3/4 inches disposable Pasteur pipets One box contains 360 pipets. Optional.	35	1	35
Glass Cutter	Cut pulled glass pipette at a point with a certain diameter, used for embryo washing and transfer from one dish to another. Optional.	Free	1	0

Cat # CCK-OPU-IVF 01: \$947/kit plus S&H Complete kit

Cat # CCK-OPU-IVF 02: \$487/kit plus S&H w/o RLI CR 01, RLI Mp-Set, disposable glass pipettes

4. Calculation of the cost per embryo produced with CK-OPU-IVF 01

CK-OPU-IVF 01 is designed for **four times** of OPU-IVF production **over a two- three week working period**. The price of CMK-OPU-IVF 01 is \$622/kit, CCK-OPU-IVF 01 with additional/optional consumables as one set of Cumulus Remover (RLI CR 01), one set of Cumulus Removal Glass (RLI CRG 01), Mouth Piece Set (RLI Mp-Set) and one box of Fisher band glass pipettes costs \$947/kit. After first order, the price of CCK-OPU-IVF 02 is only \$487/kit. Therefore, after the first order, **the price of CK-OPU-IVF 01 is \$1109/kit.**

Each SAR IVF 01 package contains Stock I (SAR IVF S-I), 1×100 mL/bottle; Stock II (SAR IVF S-II), 2×800 μL/tube; Stock II (SAR IVF S-II), 2×770 μL/tube. Because each IVF uses 20 mL of sperm washing medium and 5 mL of fertilization medium (25 mL), one package can be used for four IVF trials. For each IVF trail, two Petri dishes are prepared with 7 droplets per dish for IVF. If 20 oocytes are added into each fertilized droplet total of 280 oocytes ($20 \times 7 \times 2 = 280$) can be fertilized. As a result, one package of SAR IVF 01 can fertilize 1120 ($280 \times 4 = 1120$) oocytes.

During one trial of OPU-IVF, if 5 donors are aspirated by OPU, each 20 oocytes per donor animal are collected for maturation, subsequently for IVF, 100 oocytes can be collected from one session of OPU. If 25% oocytes develop into transferable blastocysts, 5 fresh and transferable embryos are produced from each donor per session, and total 25 (5 embryos/donor × 5 donors = 25) blastocysts are generated from a single OPU-IVF trial. As a result, 100 transferable blastocysts are produced in four OPU-IVF trails with one kit of CK-OPU-IVF 01.

The basic cost for producing one embryo, in one OPU-IVF trial with 5 donors assigned, is calculated as \$11.09 (1109/100 embryos=11.09).

If ten donors are aspirated for one OPU-IVF trail, the cost is further reduced as \$5.54 per embryo.

CK-OPU-IVF 01 is an affordable and effective kit for your OPU/IVF embryo production.