COSMO BIO CO., LTD.

HTF （for *in vitro* fertilization）

Cat. No. CSR-R-B070

CSR-R-B071

www.cosmobio.com

* Keep them at 4℃ until use. Use all the media once opened and avoid using the remaining residue as it is not so stable for repetitive use.

A: Superovulation induction

Induce superovulation in mature female mice (8-12 weeks old) by intraperitoneal administration of PMSG and hCG (7.5 IU/one mouse) at 48-hour intervals (generally, treatment of hormone administration should be operated in the period 17:00-18:00)

B: Preparation of drops

Prepare drops of culture media and equilibrate them in CO_2 incubator overnight as described below.

- For sperm collection
  Prepare 150 μL drops on a dish and cover them with liquid paraffin.
- For oocyte collection
  Prepare 200 μL and 100 μL drops on a dish and cover them with liquid paraffin.

C: Collection of sperm

1. Disinfect all dissectors with alcohol before operation.
2. Euthanize 1-2 of male mouse and pull out epididymis, testicle and part of fat using scissors and forceps. Cut out only the tail of epididymis on a filter paper, and remove blood or fats.
3. Fix the tail of epididymis with forceps and incise duct of epididymis, center part of the tail with Noyes scissors
4. Collect pellets of sperm from the duct of epididymis, incised by pressing slightly on the surface of tail of epididymis
5. Take out pellets of sperm with dissecting needle and apply to the drops on the dish.
6. Deliver the pellets of sperm exposed to HTF and incubate in CO_2 incubator（37℃ 5% CO_2 95% air）for 90 minutes.

D: Collection of oocyte

1. Disinfect all dissectors with alcohol before operation.
2. Euthanize a female mouse after treatment of superovulation and pull out the uterus, oviduct, ovary, and part of fat using scissors and forceps. Cut out only oviduct on a filter paper, and remove blood or fats.
3. Drop the oviduct in the liquid paraffin prepared for oocyte collection as described in B.
4. Fix the oviduct with forceps holding it on the bottom of dish for oocyte collection and tear the wall of oviduct with dissecting needle and apply the pellets of oocyte to 200 μL drop on the dish prepared in B .
5. After collection store the dish in CO_2 incubator.

E : Insemination – Check of fertilization

1. Draw up 2 μ L of pre-cultured sperm suspension from the dish for sperm collection and add to the dish for oocyte collection.
   * volume of sperm suspension should be adjusted depending on its motility or concentration
2. Culture the dish for fertilization prepared in 1 in incubator（37℃ ,5% CO_2 95% air）.
3. After 5-6 hours from insemination process, pick up oocytes (morphologically normal ones) with glass capillary, transfer them to 100 μ L drop on the dish for oocyte collection.
4. Observe oocytes carefully, remove parthenogenetic developed ones and culture until the next day.
5. After 24-28 hours from insemination process, count the number of 2-cell stage embryos for transfer or cryopreservation. 2-cell stage embryos collected can be cultured *in vitro* until blastocyst stage with KSOM.

COSMO BIO CO., LTD.
TOYO EKIMAE BLDG. 2-20, TOYO 2-CHOME,
KOTO-KU. TOKYO 135-0016, JAPAN
TEL : (81)3-5632-9617
FAX : (81)3-5632-9618
e-mail : export@cosmobio.co.jp
URL : www.cosmobio.com