COSMO BIO USA.

PB1, M2 (for *in vitro* culture of Mouse or Rat Embryos)

ARK Resource 生命の進化と多様性を考える
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Cat. No. ARK-R-M083, ARK-R-M084
ARK-R-P138, ARK-R-P185

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*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.

【Protocol for mouse subjects】

1-A : Superovulation induction and mating

Induce superovulation in mature female mice (8–12weeks old) by intraperitoneal administration of PMSG and hCG (7.5 IU/one mouse) at 48–hour intervals.
In case using method of perfusion flushing in oviduct, it becomes important to confirm existence of mating. Let the male mice live together with subject of female ones and check vaginal plugs on the next day. If mating is confirmed, pronuclear–stage embryos can be collected on the same day and 2–cell–stage embryos can be on the next day by perfusion. In the case of *in vitro* fertilization, collect embryos from ampulla of oviduct and fertilize them. (Please refer to the datasheet of HTF (#ARK–R–B070, #ARK–R–B071) for *in vitro* fertilization)

1-B : Preparation of drops

Place 3 drops of KSOM (100µL each) into a dish and cover them with liquid paraffin.
Incubate (5%CO_2) for at least 30 minutes to equilibrate with gas.

1-C : Collection of embryos (perfusion flushing in oviduct)

Disinfect all dissectors with alcohol before operation and heat PB1 for flushing without the process of equilibrating.

1. Euthanize a female mouse confirmed its mating and pull out the uterus, ovary, and part of fat using scissors and forceps. Cut out only the oviduct on a filter paper, and remove blood or other junk materials.
2. Insert glass capillary or flush needle to fimbria of the collected oviduct, and flush the PB1 (or M2) for perfusion.
3. Transfer the embryos into the KSOM drops previously described in 1–B.
   * 2–cell–stage emryos collected are possible to apply their culture *in vitro* until the stage of blastcyst without medium exchange.

【Protocol for rat subjects】

2 -A : Superovulation induction and mating

Induce superovulation in mature female mice (8–12weeks old) by intraperitoneal administration of PMSG (150 IU/one Wister rat) at 48–hour intervals.
In case using method of perfusion flushing in oviduct, it becomes important to confirm existence of mating. Let the male mice live together with subject of female ones and check vaginal plugs on the next day. If mating is confirmed, pronuclear–stage embryos can be collected on the same day and 2–cell–stage embryos can be on the next day by perfusion.

2 -B : Preparation of drops

Place 3 drops of mR1ECM (100 µL each) into a dish and cover them with liquid paraffin.
Incubate (5%CO_2) for at least 30 minutes to equilibrate with gas.

2 -C : Collection of embryos (perfusion flushing in oviduct)

Disinfect all dissectors with alcohol before operation and heat PB1 for flushing without the process of equilibrating.

1. Euthanize a female mouse confirmed its mating and pull out the uterus, ovary, and part of fat using scissors and forceps. Cut out only the oviduct on a filter paper, and remove blood or other junk materials.
2. Insert glass capillary or flush needle to fimbria of the collected oviduct, and flush the PB1 (or M2) for perfusion.
3. Transfer the embryos into the mR1ECM drops previously described in 2–B.
   * 2–cell–stage embryos collected are possible to apply their culture until the stage of blastcyst *in vitro* with mR1ECM (400µL/drop)

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