

Rat KSOM (*in vitro* culture of rat embryos)

Cat. No. CSR-R-148

Size $10 \times 1 \text{ ml}$

Last updated: 2024/6/18

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*Keep them in 4° C until use. Use all the media once opened and avoid using the remaining because of the quality degradation.

Superovulation induction and mating

Mature female Wistar rats (8-12 weeks old) received intraperitoneal administration of PMSG (150 IU/ kg/rat) and hCG (75 IU/kg/rat) at 48-hour intervals to induce superovulation. After hCG administration, they were mated with male rats. On the next day, vaginal plugs were confirmed to ensure mating. Mated female rats were used in the experiment. Pronuclear-stage embryos can be collected on the day of vaginal plug confirmation, and 2-cell-stage embryos on the next day.

Recommended schedule

PMSG: 9:00-11:00
hCG: 9:00-11:00 (48 hours after injecting PMSG)
Collection time
Pronuclear-stage fertilized eggs: 13:00-16:00 (on the day of vaginal plug confirmation)
2-cell-stage embryos: 13:00-18:00 (on the next day of vaginal plug confirmation)

Preparation of drops

Place 3 drops of Rat KSOM (100 μ L each) into a dish. Cover them with liquid paraffin and incubate (5% CO₂) for at least 30 minutes for gas equilibrium.

Collection of embryos

Disinfect all dissectors with alcohol.

Heat culture medium for flush (M2, Cat No. CSR-R-M083,084) to $37^\circ\!C$.

- 1. Euthanize a mature female rat. Pull out the uterus, ovary, and part of fat using scissors and forceps. Cut only the fallopian tube on a filter paper, and remove blood.
- 2. Insert glass capillary or flush needle to fimbria of the collected oviduct, and flush the culture medium.

* If you collect Pronuclear-stage embryos, please add hyaluronidase (final concentration: 0.1%) to the culture medium for flushing (M2).

- 3. Transfer the embryos into the Rat KSOM drops.
- 4. The resulting two-cell stage embryos are subjected to medium exchange every 24 hours until the blastocyst stage.



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