COSMO BIO CO., LTD.

CARD MEDIUM®

Cat. No. KYD-003-EX Size: 1 KIT

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www.cosmobiousa.com

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Center for Animal Resources and Development

Instruction manual of CARD MEDIUM®

CARD MEDIUM® is a novel medium which helps attain a high fertilization rate in mice, especially when used in combination with FERTIUP® pre-incubation medium.
CARD MEDIUM® was developed by The Center for Animal Resources and Development (CARD), Kumamoto University, Japan.

Contents

- An ampoule including medium (A)
- A vial including powder (B)
- A 1.5 mL plastic tube (C)
- A 1.5 mL plastic tube (D)
- A 2.5 mL disposable syringe
- A needle
- A filter unit (pore size: 0.22 µm)

Storage

Store at 4°C. Please see the labels on the ampoule (A) and the vial (B) for the use-by date.

Note

For research use only. Not for human or medicinal use.

For research use only, Not for diagnostic use.
Please read this manual thoroughly before use.

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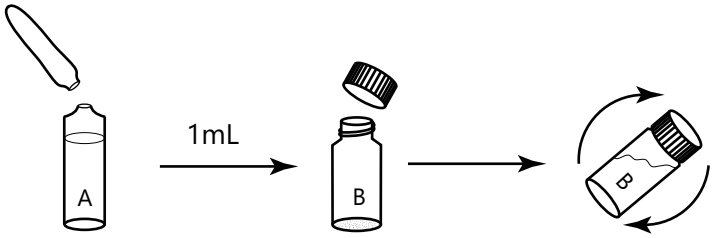
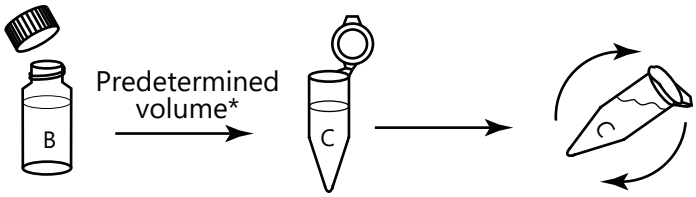
Preparation of CARD MEDIUM® for fertilization

1. Take 1 mL of the medium from the ampule (A) and add it into the vial (B). Close the lid, then mix the medium and the powder in the vial.



2. Take 1.5 mL of the medium from the ampule (A) and add it into the tube (C).

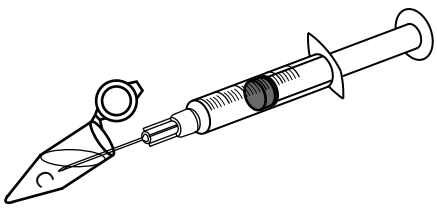
3. Put predetermined volume* from the vial (B) into the tube (C) and mix them.



Sperm	Predetermined Volume*
Frozen-thawed	15 µL
Cold stored	7.5 µL
Fresh	3.75 µL

(To make fertilized oocytes which are tolerant towards cryopreservation, use the predetermined volume of 2.25µL.)

4. Connect the needle to the syringe and suck up all of the medium in the tube (C).



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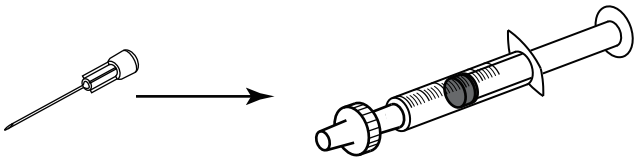
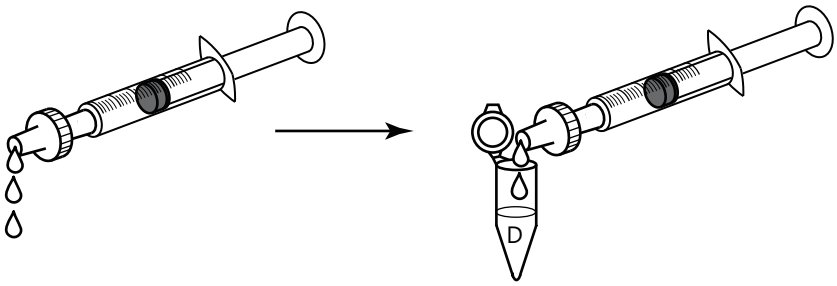
5. Remove the needle from the syringe and connect the filter unit to the syringe.

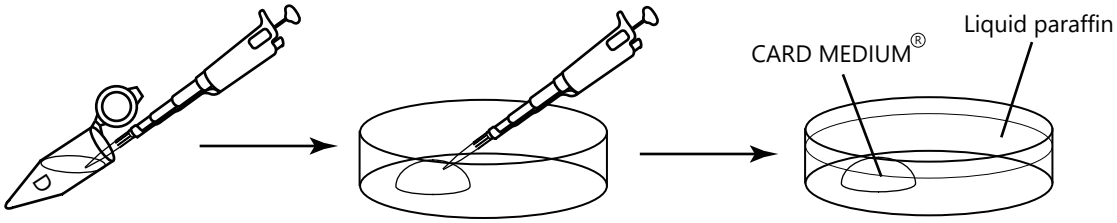


6. Pushing the syringe in, discard 2~3 drops of medium and inject the remaining medium into the tube (D).



7. Make a drop* of the medium in the tube (D) in a fertilization dish, then keep the dish in an incubator (37°C, 5% CO^2 in air) for 10 minutes.

   * A drop volume of CARD MEDIUM® for fresh and cold stored sperm: 200 µL
     A drop volume of CARD MEDIUM® for frozen-thawed sperm: 90 µL



8. Introduce oocytes from superovulated female mice into the drop of CARD MEDIUM, then add preincubated sperm into the drop to fertilize them.

   Note: Once the ampoule (A) is opened, the medium within cannot be stored.
   Moreover, once the medium has been used to dissolve the powder in the vial (B), the medium cannot be stored, even if diluted or filtered. Please use the medium within 4-5 hours of step 1).

   Comment: FERTIUP® is a recommended medium for the preincubation of sperm.

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References

For information on cryopreservation techniques for mouse spermatozoa, or on techniques used in thawing, *in vitro* fertilization and obtaining pups through embryo transfer, please refer to CARD website below.

http://card.medic.kumamoto-u.ac.jp/card/english/sigen/index.html

References

1) Takeo T., Hoshii T., Kondo Y., Toyodome H., Arima H., Yamamura KI., Irie T., and Nakagata N. 2008. Methyl-beta-cyclodextrin improves fertilizing ability of C57BL/6 mouse sperm after freezing and thawing by facilitating cholesterol efflux from the cells. ***Biol Reprod.*** 78(3): 546-51.

2) Takeo T., and Nakagata N. 2010. Combination medium of cryoprotective agents containing L-glutamine and methyl-β-cyclodextrin in a preincubation medium yields a high fertilization rate for cryopreserved C57BL/6J mouse sperm. ***Lab Anim.*** 44(2): 132-7.

Note

*FERTIUP® was jointly developed by KYUDO CO., LTD. and Kumamoto University.

*FERTIUP® is a trademark of KYUDO CO., LTD.

*FERTIUP® PATENT Number JP4683408

*CARD MEDIUM® are based on the results of research in The Center for Animal Resources and Development (CARD), Kumamoto University, Japan.

*CARD MEDIUM® is a trademark of KYUDO CO., LTD.

Manufacturer
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