

## Instruction manual

CARD MEDIUM<sup>®</sup> is a novel medium which helps attain a high fertilization rate *in vitro* in mice, especially when used in combination with FERTIUP<sup>®</sup> pre-incubation medium.

CARD MEDIUM<sup>®</sup> 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 1.5mL plastic tube (C)

A 1.5mL plastic tube (D)

A 2.5mL disposable syringe

A needle

A filter unit (pore size: 0.22  $\mu$ m)

### Storage

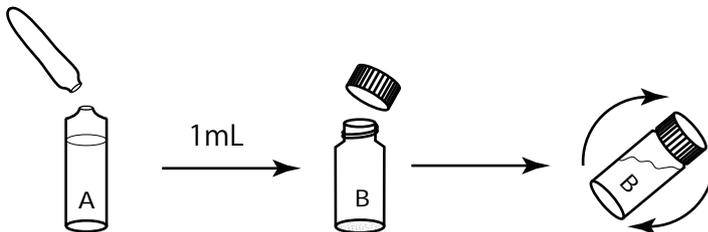
Store at 4 °C. Please see the labels on the ampoule (A) and the vial (B) for the use-by date.

### Note

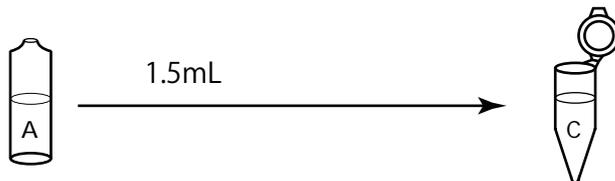
For research use only. Not for human or medicinal use.

### Preparation of CARD MEDIUM<sup>®</sup> for *in vitro* fertilization

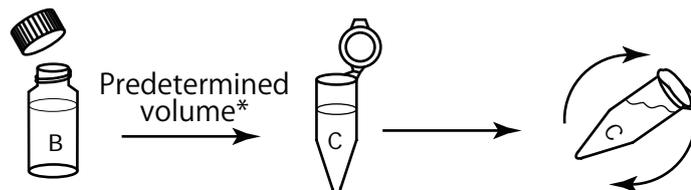
- 1) Take 1mL of the medium from the ampoule (A) and add it into the vial (B). Close the lid, then mix the medium and the powder in the vial.



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

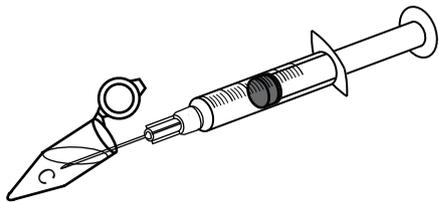


- 3) Put predetermined volume\* from the vial (B) into the tube (C) and mix them.

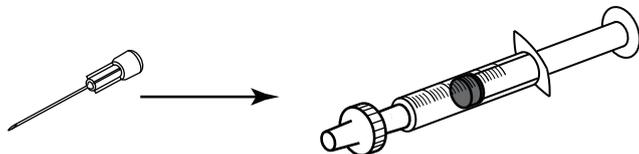


Sperm	Predetermined Volume*
Frozen-thawed	15 $\mu$ L
Cold stored	7.5 $\mu$ L
Fresh	3.75 $\mu$ L

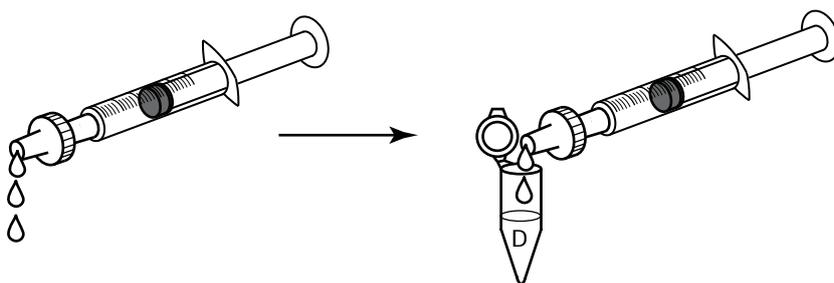
4) Connect the needle to the syringe and suck up all of the medium in the tube (C).



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

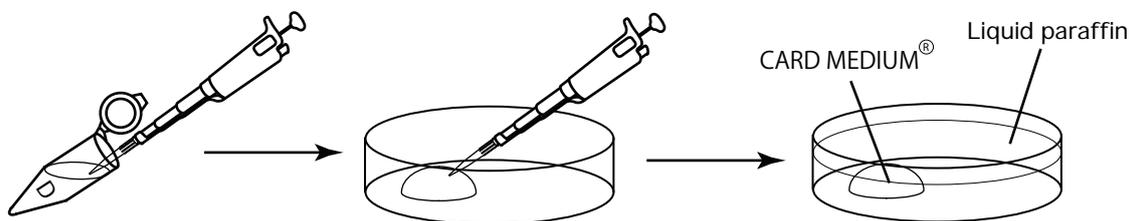


6) Pushing the syringe in, discard 2~3 drops of medium and inject the remaining medium into the tube (D).



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<sub>2</sub> in air) for 10 minutes.

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



8) Introduce oocytes from superovulated female mice into the drop of CARD MEDIUM<sup>®</sup>, 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<sup>®</sup> is a recommended medium for the preincubation of sperm.

\*CARD MEDIUM<sup>®</sup> is a trademark of Kumamoto University

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

\*FERTIUP<sup>®</sup> is a trademark of KYUDO CO., LTD.

\*FERTIUP<sup>®</sup> was jointly developed by KYUDO CO., LTD. and Kumamoto University.

\*FERTIUP<sup>®</sup> PATENT Number JP4683408

Manufacturer



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