

## R18S3 (medium for mouse sperm cryopreservation)



Cat. No. ARK-R-R082

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\*Store at room temperature.

Use up soon after opening, due to keeping the product quality.

## Freezing

- 1. Pick up the cauda epididymis from male C57BL/6J mouse, remove adherent adipose tissue and blood, and put two cauda epididymis into 100  $\mu$ L of R18S3 on a petri dish.
- 2. Fix the cauda epididymis with forceps and cut the ductus epididymis about ten times. After that, shake the petri dish for 1 min and suspend medium to make sperm equal.
- 3. Connect the straw with 1 mL syringe. Sealing protocol is as follows.
  - Aspirate 100 µL of HTF (used as a weight) and prepare 1 cm airspace at the edge of straw.
  - Use above syringe and aspirate 10  $\mu L$  of sperm-suspension prepared at step (2). And then prepare 1 cm airspace at the edge of straw.
  - Seal the edge of straw with heat sealer.
  - Detach the straw from the syringe and seal another edge of straw.
- 4. Put the sealed straw into float, with turning sperm-suspension layer to the bottom. Put the straw-float on the surface of liquid nitrogen in N2 tank. Keep it stand for 15 min.
- 5. After 15 min, soak it into liquid nitrogen.

## **Thawing**

- 1. Pick up the freezing straw from liquid nitrogen, and soak it into warm water at 37  $^{\circ}$ C . Keep it stand for 15 min.
- 2. After 15 min, pick up the straw from warm water and wipe it with some papers. After that, cut the both edges of straw and drop the sperm-suspension on the petri dish.
- 3. Pick up 2  $\mu$ L of sperm-suspension and apply it into 200  $\mu$ L HTF, which is pre-warmed in incubator (37 °C , 5% CO2 in air) beforehand.
  - After 1 hour from above treatment, observe the motility and active level.





COSMO BIO USA

2792 Loker Ave West, Suite 101
Carlsbad, CA 92010, USA
email: support@cosmobiousa.com
URL: www.cosmobiousa.com
Phone: (+1) 760-431-4600