COSMO BIO CO., LTD.

R18S3
(medium for mouse sperm cryopreservation)

Cat. No. CSR-R-R082 **Size:** 10 *1 ml
(1ml *5vial *2box)

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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 µL 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 µL 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 N_2 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 ℃ . 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 µL of sperm-suspension and apply it into 200 µL HTF, which is pre-warmed in incubator (37 ℃ , 5% CO_2 in air) beforehand. After 1 hour from above treatment, observe the motility and active level.

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