

## P10 and PEPeS (Media for cryopreservation)

Cat. No. CSR-R-P186

CSR-R-P187

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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.

## Collection of embryos

Collect the embryos of the required stage by oviduct perfusion after mating (Please refer to the datasheet of mR1ECM (Cat No. CSR-R-M174 or CSR-R-M191).

## Preparation

- 1. Return P10 to room temperature.
- 2. Prepare cryotubes depending on the number of embryos you freeze.
- 3. Prepare crushed ice, chiller or lab top cooler to keep samples to 0℃. Cool PEPeS to 0℃.
- 4. Prepare cryobox or cryocanes according to the number of tubes you freeze. (Cryotubes will be kept in liquid nitrogen.)

## Cryopreservation

- 1. Put P10 drops in a dish. The number of drops should be the number of tubes you freeze + 1.
- 2. Place embryos to one of the drops and wait until the embryo drops down.
- 3. Using glass capillary, divide the embryos equally and move them to the remaining P10 drops.
- 4. Using a pipette, transfer the embryos with 5  $\mu$ L of P10 into a cryotube.
- 5. Keep the cryotube to  $0^{\circ}$ C.
- 6. Add 95 µL of PEPeS into the cryotube along the inner surface and equilibrate for 1 minute.
- 7. Put the tubes to pre-cooled cryobox or cryocanes, and preserve them in liquid nitrogen.
- 8. Keep them into liquid nitrogen. If the samples are kept above the liquid nitrogen, they will melt and will cause the poor survival rate.



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