



KAMIYA BIOMEDICAL COMPANY

Mouse Embryo Cryopreservation Kit

For the cryopreservation and thawing of mouse embryos

Cat. No. KT-102

For research use only.

PRODUCT INFORMATION**Mouse Embryo Cryopreservation Kit**

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PRODUCT

The Mouse Embryo Cryopreservation Kit is a set of culture media, cryopreservation solution and thawing solution for mouse embryos. Kit is suitable for cryopreserving about 8,000 mouse embryos.

COMPONENTS**For Cryopreservation**

Component	Main Ingredients	Size
TYH Medium	NaCl, KCl, CaCl ₂ , KH ₂ PO ₄ , MgSO ₄ , NaHCO ₃ , Glucose, Na-Pyruvate, Penicillin, Streptomycin, BSA	5 mL x 1
mW Medium	NaCl, KCl, KH ₂ PO ₄ , MgSO ₄ , NaHCO ₃ , Ca-lactate, Glucose, Na-Pyruvate, Penicillin, Streptomycin, Phenol Red, β-Mercaptoethanol, EDTA, BSA	5 mL x 1
PB1 Solution	NaCl, KCl, CaCl ₂ , KH ₂ PO ₄ , MgCl ₂ , Na ₂ HPO ₄ , Glucose, Na-Pyruvate, Penicillin, BSA	5 mL x 1
1M DMSO Solution	DMSO	2 mL x 1
DAP213 Preservation Solution	PB1 + DMSO, acetamide, propylene glycol	2 mL x 1

For Thawing

Component	Main Ingredients	Size
0.25M Sucrose Solution	Sucrose	2 mL x 5
mW Medium	NaCl, KCl, KH ₂ PO ₄ , MgSO ₄ , NaHCO ₃ , Ca-lactate, Glucose, Na-Pyruvate, Penicillin, Streptomycin, Phenol Red, β -Mercaptoethanol, EDTA, BSA	5 mL x 5

PROTOCOLS***In vitro* Fertilization and Culturing of Embryos****1. Materials and equipment**

- TYH Medium
- mW Medium
- Mineral oil
- Capillary pipette (homemade from a glass hematocrit tube)
- Petri plate, plastic, 35 mm diameter
- Micropipette
- Pipet tips
- Surgical instrument
- Stereoscopic microscope
- CO₂ incubator

2. Procedure for *in vitro* fertilization

1. One day before the experiment, prepare 200 μL drops of TYH Medium on plastic petri plates. Cover them with mineral oil, and place the plates in a CO_2 incubator.
2. On the day of experiment, collect sperm from the testis of male mice and pre-culture them in TYH Medium for 1 hour.
3. Remove the oviduct from female mice which have been treated with gonadotropin to induce ovulation. Place it under mineral oil in the pre-equilibrated medium drops.
4. Under the stereoscopic microscope, tear the enlarged part of the oviduct with a surgical needle under mineral oil to remove the cluster of eggs into TYH Medium.
5. To the TYH Medium containing eggs, add precultured sperm suspension so that the final sperm concentration is 150 sperm/ μL .
6. After insemination, immediately place the petri plate in a CO_2 incubator to culture further.

3. Procedure for embryo culture

1. Prepare 3 drops of 100 μL mW Medium per petri plate. Cover them with mineral oil.
2. Place them in a CO_2 incubator to equilibrate.
3. Wash fertilized eggs obtained by *in vitro* fertilization by transferring them to the mW Medium drops three times. Return to a CO_2 incubator to continue the culture.

Cryopreservation of Embryos

1. Materials and equipment

- PB1 Solution
- 1M DMSO Solution
- DAP213 Preservation Solution
- Capillary pipette
- Petri plate, plastic, 35 mm diameter
- Cryotubes (1 mL, Nunc 366656)
- Micropipette
- Pipet tips
- Stereoscopic microscope
- CO_2 incubator
- Holder kane

2. Cryopreservation procedure

1. Wash the embryos by transferring 3 times to a PB1 Solution drop. Keep them in a CO_2 incubator.
2. Prepare two drops of 100 μL 1M DMSO Solution in a plastic petri plate and keep them at room temperature.
3. Place the embryos in one of the drops. The embryos initially float, but sink in 1-2 minutes. At that point, transfer them to another drop.
4. Dispense to each cryotube 20 embryos in 5 μL 1M DMSO ^{*1} and place the tubes in an aluminum block precooled to 0°C that is attached to a cooling apparatus. ^{*2} (For example, the Eppendorf ThermoStat Plus with appropriate thermoblock to fit the cryotube. However, any apparatus that cools the cryotube to 0°C is suitable.)
5. After cooling for 5 minutes, add 95 μL /tube of DAP213 Preservation Solution that has been precooled at 0°C .
6. Let them stand for another 5 minutes. Promptly place the cryotubes in holder kanes and freeze directly in liquid nitrogen.

*1 The number of embryos per cryotube can be determined arbitrarily. Twenty embryos or its multiples per tube is convenient when embryos, after thawing, are transplanted to the surrogate female mouse.

*2 The equilibration in 1M DMSO Solution and DAP213 Preservation Solution for up to 30 minutes does not affect the viability of the embryos after thawing.

Thawing of Embryos

1. Materials and equipment

- mW Medium
- 0.25M Sucrose Solution
- Capillary pipette
- Petri plate, plastic, 35 mm diameter
- Micropipette
- Pipet tips
- Stereoscopic microscope
- CO₂ incubator

2. Thawing Procedures

1. Remove the cryotubes from liquid nitrogen tank. Remove the caps and let the tubes stand at room temperature for 90 seconds.
2. Add to each tube 900 µL of 0.25M Sucrose Solution preheated at 37°C and mix by pipetting up and down with micropipette.
3. Transfer the content of tube to a Petri plate. Collect embryos under the stereoscopic microscope and wash by transferring 3 times to fresh mW Medium.
4. Return the petri plate to a CO₂ incubator and let it stand for 10 minutes. Inspect the embryos for normal morphology. The cells of dead embryos are broken or their cell membrane is shrunk and dark.

STORAGE

Store at 4°C. The kit is stable until the expiration date indicated on the label when stored at 4°C. Do not freeze.

FOR RESEARCH USE ONLY

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