

Introducing reagents for use in rat reproductive engineering

- CARD FERTIUP® Rat Sperm Cryopreservation
- CARD Rat IVF medium
 - A medium for rat in vitro fertilization -
- CARD Rat sperm freezing kit



Massive improvements in rat strain preservation efficiency and live animal maintenance costs through rat sperm cryopreservation!

Manufacturer



Technical Support :
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Distributor



Distributed worldwide by Cosmo Bio USA

COSMO BIO USA

Great Reagents Drive Great Research

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- **Are you having trouble with the preservation of genetically modified rat strains?**
- **Are you worried about the cost of maintaining and managing these rat strains?**

Then rat sperm cryopreservation techniques could be the answer to your problems!

Try CARD reagents for rat reproductive technology.

Rat sperm cryopreservation techniques - Development background

Rats are widely used in medical and life science research. In recent years, various strains of genetically modified rat have been produced worldwide thanks to the development of genome editing technologies. Moreover, it is predicted that even more genetically modified rats will be produced in the future. Therefore, there is a pressing need for effective preservation techniques for these genetically modified rat strains.

Through sperm freezing, it is possible to quickly cryopreserve a large number of sperm taken from the cauda epididymis of a male rat. For this reason, sperm cryopreservation is expected to be an easier, more effective and most economically viable technique for the preservation of genetically modified rat strains than embryo cryopreservation. However, rat sperm is vulnerable to the effects of physical changes to pH, osmotic pressure, temperature and other similar factors. Moreover, it is difficult to maintain the motility of frozen-thawed sperm via conventional sperm cryopreservation methods, which means that it is not viable to use these sperm in in vitro fertilisation (IVF). Therefore, the establishment of rat sperm cryopreservation and rat IVF techniques was required to solve these issues.

A research team led by Dr. Naomi Nakagata and Dr. Toru Takeo at the Institute of Resource Development and Analysis, Kumamoto University have developed a practical rat sperm cryopreservation technique and an IVF method using frozen-thawed rat sperm. These techniques may offer a solution to the problem of genetically modified rat strain preservation.

Reagents and equipment for use in rat reproductive engineering

CARD FERTIUP® Rat Sperm Cryopreservation Agent

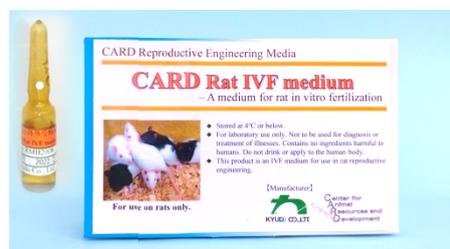
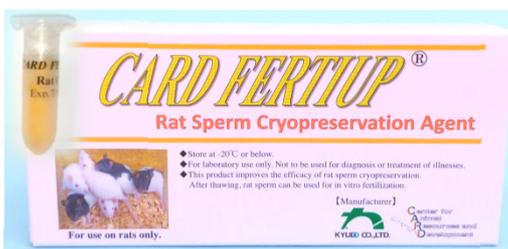
CARD FERTIUP® Rat Sperm Cryopreservation Agent is a newly-developed agent which enables researchers to efficiently carry out cryopreservation of rat sperm, which until now has been notoriously difficult to cryopreserve. Conventional rat sperm cryopreservation methods are unable to effectively maintain the motility of frozen-thawed sperm. On the other hand, rat sperm cryopreservation carried out using CARD FERTIUP® Rat Sperm Cryopreservation Agent yields superior motility even in frozen-thawed rat sperm, allowing for the production of many rat pups via IVF and embryo transfer.

CARD Rat IVF medium

CARD Rat IVF Medium is a new IVF medium developed specially for use in rat IVF.

CARD Rat sperm freezing kit

CARD Rat Sperm Freezing Kit is a kit that facilitates the effective production of high-quality frozen rat sperm during rat sperm cryopreservation using CARD FERTIUP® Rat Sperm Cryopreservation Agent. With this kit, researchers can appropriately carry out the rat sperm cooling process during rat sperm cryopreservation.



Characteristics and effectiveness

Rat sperm cryopreservation using CARD FERTIUP® Rat Sperm Cryopreservation Agent and rat IVF carried out using frozen-thawed sperm are revolutionary techniques which have enabled the production of high quality frozen rat sperm and the acquisition of high IVF efficiency rates using frozen-thawed rat sperm at a practical level for the very first time. The fertilised oocytes obtained via IVF develop well, and after transferring embryos to a female rat, one can efficiently acquire genetically modified rat pups. (see Table 1, Table 2 and Fig. 1)

Usage examples / benefits

1. Implementation of high quality IVF using frozen-thawed rat sperm
2. Storage of genetically modified rat strains for subsequent use in the production of pups
3. Massive reduction in rat maintenance costs through effective rat strain preservation

| Sperm | No. | No. of inseminated oocytes | No. of offertilised oocytes | Fertilisation rate (%) |
|--------|--------------|----------------------------|-----------------------------|------------------------|
| Fresh | 1 | 198 | 185 | 93.4 |
| | 2 | 183 | 170 | 92.9 |
| | 3 | 195 | 181 | 92.8 |
| | 4 | 182 | 174 | 95.6 |
| | Total | 758 | 710 | 93.7±1.3 |
| Frozen | 1 | 161 | 135 | 83.9 |
| | 2 | 191 | 160 | 83.8 |
| | 3 | 201 | 161 | 80.1 |
| | 4 | 196 | 159 | 81.1 |
| | Total | 749 | 615 | 82.1±1.9 |

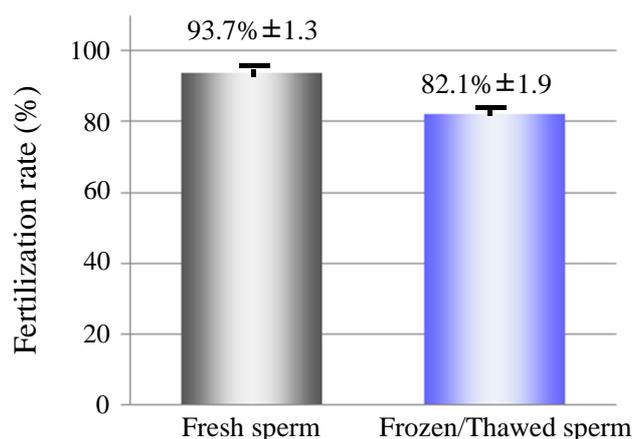
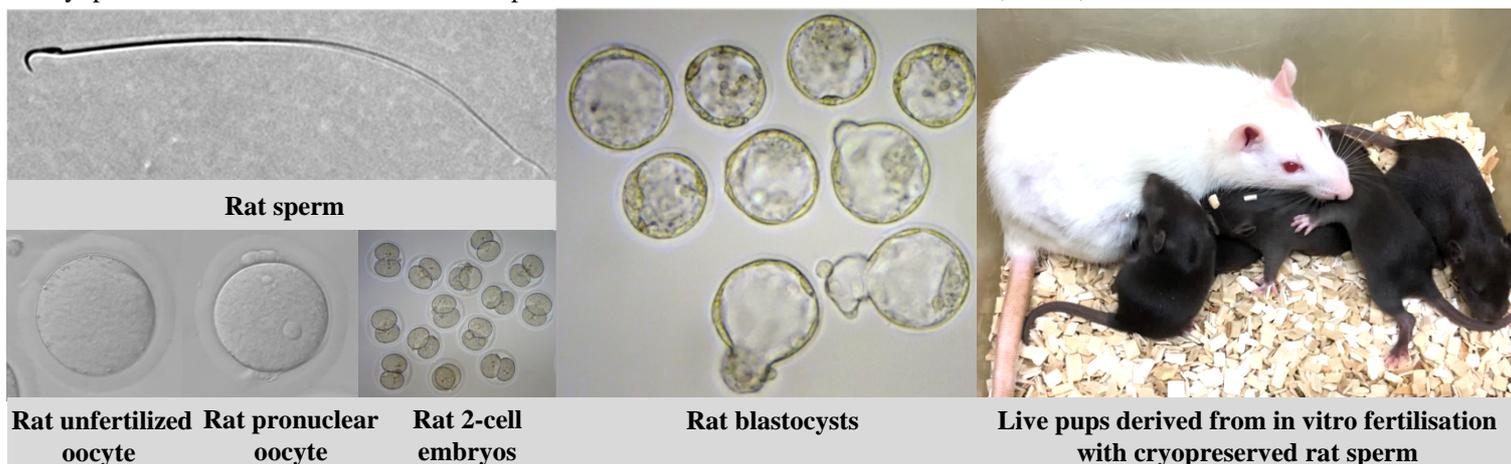


Table1 / Fig.1 IVF fertilisation rates using fresh and frozen-thawed rat sperm.

| Sperm | No. | No. of 2-cell embryos | No. of blastocysts | Developmental rate(%) | Fertilised oocytes transferred | No. of pups | Birth rate(%) |
|--------|--------------|-----------------------|--------------------|-----------------------|--------------------------------|-------------|-----------------|
| Fresh | 1 | 100 | 58 | 58 | 20 | 13 | 65.0 |
| | 2 | 100 | 63 | 63 | 20 | 12 | 60.0 |
| | 3 | 100 | 65 | 65 | 20 | 13 | 65.0 |
| | 4 | 100 | 71 | 71 | 20 | 10 | 50.0 |
| | Total | 400 | 257 | 64.3±5.4 | 80 | 48 | 60±7.1 |
| Frozen | 1 | 100 | 72 | 72 | 20 | 12 | 60.0 |
| | 2 | 100 | 57 | 57 | 20 | 12 | 60.0 |
| | 3 | 100 | 64 | 64 | 20 | 10 | 50.0 |
| | 4 | 100 | 53 | 53 | 20 | 12 | 60.0 |
| | Total | 400 | 246 | 61.5±5.4 | 80 | 46 | 57.5±5.0 |

Table2 Rate of development of fertilised oocytes derived from fresh and frozen-thawed rat sperm to the blastocyst stage, and birth rate of said fertilised oocyte after oocyte transfer

Reference : Naomi Nakagata, Nobuyuki Mikoda, Satohiro Nakao, Ena Nakatsukasa & Toru Takeo, Establishment of sperm cryopreservation and *in vitro* fertilisation protocols for rats. SCIENTIFIC REPORTS, 10:93, 2020.



The above data and photographs are published with the permission of Kumamoto University.

Reproductive Engineering Reagent List

| Code No. | Reagents for use in rat reproductive engineering | STANDARD |
|-------------|---|------------|
| KYD-FR-001 | NEW CARD FERTIUP [®] Rat Sperm Cryopreservation | 1 × 1.5 mL |
| KYD-FR-002 | NEW CARD Rat IVF medium - A medium for rat in vitro fertilization - | 1 × 1.0 mL |
| KYD-GAR-001 | NEW CARD Rat sperm freezing kit | 1 kit |

| Code No. | Reagents for use in mouse reproductive engineering | STANDARD |
|------------------|--|------------|
| KYD-001-05-EX | CARD FERTIUP [®] Mouse Sperm Cryoprotectant : CPA | 1 × 0.5 mL |
| KYD-001-05-EX-X5 | | 5 × 0.5 mL |
| KYD-001-EX | | 1 × 1.0 mL |
| KYD-001-EX-X5 | | 5 × 1.0 mL |
| KYD-002-05-EX | CARD FERTIUP [®] Mouse Sperm Preincubation Medium : PM | 1 × 0.5 mL |
| KYD-002-05-EX-X5 | | 5 × 0.5 mL |
| KYD-002-EX | | 1 × 1.0 mL |
| KYD-002-EX-X5 | | 1 × 1.0 mL |
| KYD-003-EX | CARD MEDIUM [®] Mouse Fertilization Medium | 1 kit |
| KYD-004-EX | CARD FERTIUP [®] PM (1.0 mL) and CARD MEDIUM [®] | 1 set |
| KYD-005-EX | CARD FERTIUP [®] PM (0.5 mL) and CARD MEDIUM [®] | 1 set |
| KYD-010-06-EX | CARD HyperOva [®] Superovulation Reagent for Mice | 1 × 0.6 mL |
| KYD-010-06-EX-X5 | | 5 × 0.6 mL |
| KYD-010-EX | | 1 × 1.0 mL |
| KYD-010-EX-X5 | | 5 × 1.0 mL |
| KYD-015-EX | CARD HyperOva [®] F.D. Superovulation Reagent for Mice | 1 × 2.0 mL |
| KYD-015-EX-X5 | | 5 × 2.0 mL |
| KYD-008-02-EX | CARD mHTF | 1 × 2.0 mL |
| KYD-008-02-EX-X5 | | 5 × 2.0 mL |
| KYD-008-05-EX | | 1 × 5.0 mL |
| KYD-008-05-EX-X3 | | 3 × 5.0 mL |
| KYD-011-EX-2 | CARD KSOM | 1 × 2.0 mL |
| KYD-011-EX-5 | | 1 × 5.0 mL |
| KYD-012-EX | CARD 1M DMSO | 1 × 1.0 mL |
| KYD-013-EX-0.5 | CARD DAP213 | 1 × 0.5 mL |
| KYD-013-EX-1 | | 1 × 1.0 mL |
| KYD-014-EX-2 | CARD 0.25M Sucrose | 1 × 2.0 mL |
| KYD-014-EX-5 | | 1 × 5.0 mL |

| Code No. | Accessory for use in mouse/rat reproductive engineering | STANDARD |
|-------------|---|----------|
| KYD-006-EX | CARD Cold Transport Kit | 1 Set |
| KYD-S036 | Embryo manipulation instrument set | 1 Set |
| KYD-S018 | Freezing Canister | Unit |
| KYD-S035 | Triangular Cassette Long (10units) | 10 Units |
| KYD-S020X10 | Sperm Straws (10 Pieces x 10 Units) | 10 Units |

Note : CARD reagents for mouse/rat reproductive technology are for laboratory use only and are not to be used for the diagnosis or treatment of illnesses.