

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



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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

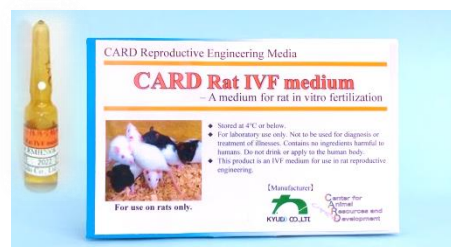
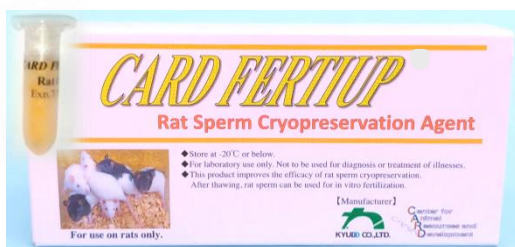
CARD FERTIUP Rat Sperm Cryopreservation Agent is a newly-developed agent which enables researchers to efficiently carry out cryopreservation of rat sperm, which until now has been notoriously difficult to cryopreserve. Conventional rat sperm cryopreservation methods are unable to effectively maintain 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 fertilised 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

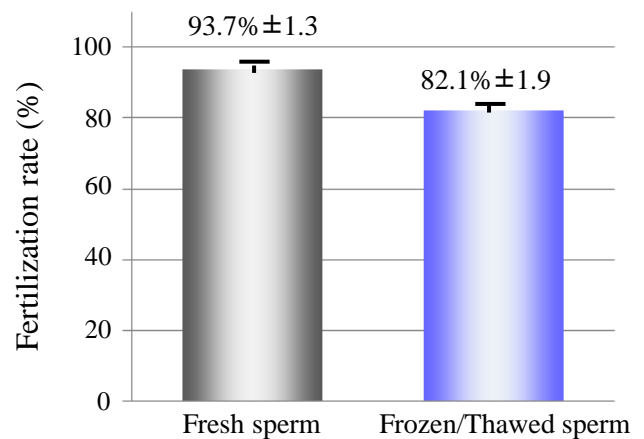
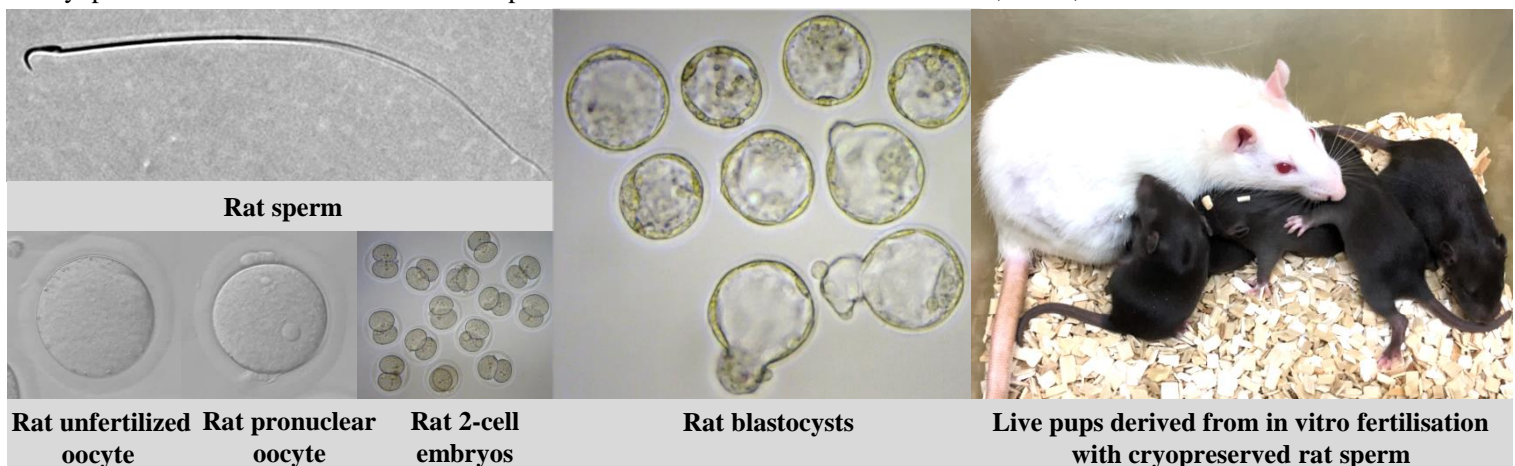


Table1 / Fig.1 IVF fertilisation rates using fresh and frozen-thawed rat sperm.

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.



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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.

※FERTIUP is our registered trademark.