胚胎玻璃化冷冻液使用说明书 （小鼠专用）

产品	货号	规格	用途
EFS40	72023	1mL	小鼠胚胎冷冻保护剂
EFS20	72024	1mL	小鼠胚胎冷冻保护剂

【主要组成成分】

DPBS、BSA、Ficoll等

【用途原理】

冷冻保护剂EFS40、EFS20配合使用，可用于小鼠胚胎冷冻保存。

【储存条件及有效期】

1、储存条件：-20℃，避光保存

2、有效期：6 个月，开口后未使用的试剂，请用封口膜密封，重新放入-20℃

【胚胎要求】

透明带完整，透明带间隙正常，胚胎形态规则，无异常

【使用方法】

1、先将EFS20、EFS40从-20 ℃冰箱中取出放于金属温控仪（0℃）中。

2、取出冻存管放入金属温控仪（0℃）中预冷。

3、将移液器调至50μL，一次性吸入50μL的EFS40，轻轻地打入冻存管底部。

4、取一个新的35mm培养皿，并用EFS40做一个任意大小的滴，EFS20做若干个50μL滴。

5、在显微镜下用移卵针收集IVF得到的所有正常发育的2 cell 胚胎（干净无杂质）。

6、放入预先准备好的EFS20的滴中，计时2min。实验中可以发现当用移卵针将胚胎放入EFS20中是悬浮着的。

7、2min时间快到时（倒数40s），用移卵针先吸入少许EFS40，做气泡，再吸入少许EFS40，紧接着一次性吸入放在EFS20中平衡的所有胚胎，并轻轻的吹入冻存管底部（看到有气泡吹进即可），放在金属金属温控仪中（0℃）计时1min后。投入液氮中。

【注意事项】

1、盖冻存管盖子时不能拧太紧以免复苏时拧不开盖子而影响操作；

2、若没有金属温控仪，可使用冰盒替代，保证 0℃环境；

3、从EFS20中吸取胚胎放进冻存管时，吸入EFS20的量应尽量少；

4、投入到液氮里时，应保证冻存管被完全浸入液氮中；

5、建议每管胚胎冻存数量50-100R/管。

Vitrification solution used for mouse embryo cryopreservatio (Mouse-specific)

Product	Item No.	specification	Application
EFS40	72023	1mL	Embryo cryoprotectant solution in mice
EFS20	72024	1mL	Sperm cryoprotectant solution in mice

Composition

DPBS、BSA、Ficoll.

Uses and Principles

The combination of EFS40 and EFS20 can be used for cryopreservation of mouse embryos.

Storage conditions and expiration date

1. Storage Temperature: -20℃,Protect from light.
2. Expiration date: 6 months, unused reagents after opening should be sealed with parafilm and Stored at -20℃.

Embryo selection:

Select embryos with normal morphology for cryopreservation.

Methods

1. Take EFS20 and EFS40 out from-20℃ been placed in a metal temperature controller (0℃).
2. Take out the cryovials and place it in a metal temperature controller (0℃).
3. Pipette 50 μL of EFS40 into the bottom of the cryovials gently.
4. Make one drop in a 35mm dish with EFS40 and several 50 μL drops with EFS20.
5. 2- cell stage embryos were collected that obtained from IVF.
6. The embryos were placed in drop of EFS20 for 2 min. It was observed that the embryos were suspended in EFS20.
7. At 80s, the pipette can be loaded with a few of EFS40,and followed by aspirating all embryos placed in the EFS20 and gently blowing into the bottom of the cryotube .you can be determined by the entry of bubbles to judge whether all embryos have entered

the cryovial, put it in a metal bath (0 ℃) for 1min. Immediately immerse the cryovials with embryos into LN_2 using a forceps.

Cautions:

1. When embryo cryopreservation, do not cover the cryovials cap too tightly to avoid wasting time when the cap cannot be unscrewed during embryo thawing.
2. Ice box can be used instead of metal bath to ensure 0℃temperature.
3. Aspirate embryos with a minimal volume of EFS20 medium.
4. Make sure that the cryotube is immersed in the LN_2.
5. It is recommended that embryos be frozen at 50-100R/ cryovials.