胚胎玻璃化复苏液使用说明书 （小鼠专用）

产品	货号	规格	用途
0.75Su	72025	1mL	小鼠胚胎复苏
0.25Su	72026	1mL	小鼠胚胎复苏

【主要组成成分】

DPBS和蔗糖

【用途原理】

本试剂主要用于小鼠胚胎冷冻的复苏，适用于卵母细胞至囊胚各时期胚胎的冷冻，一般需要配合KOSM或M16使用。

【储存条件及有效期】

1、储存条件：4℃，避光保存

2、有效期：6个月，开口后未使用的试剂，不建议再次使用

【使用方法】

1、0.25Su、0.75Su和M16于复苏前在CO_2培养箱中预热一个半小时以上，并用M16制作培养皿，覆盖矿物油在培养箱中过夜平衡。

2、准备一个35mm的新皿放在旁边。用镊子将冻存管从液氮中取出，立即打开管盖并计时30s 。

3、从培养箱中取出0.75Su,30s计数结束后，用1000μL移液器吸取900μL的0.75Su，计时时间一到立即加入冻存管中，并用移液器轻轻吹打冻存管底部，观察底部冻存液是否融化，待液体全部融化后，将冻存管内的所有液体转移到预先准备好的35mm培养皿中，再吸取400-500μL 0.75Su加入冻存管中，重复上述操作。

4、从培养箱中取出0.25Su，并再准备一个35mm的新皿，用200μL的移液器分别取50μL 0.25Su做三个滴备用。

5、将培养皿放于显微镜下，收集胚胎放入第一个0.25Su的滴中，计时1min，时间一到，收集胚胎放入第二个0.25Su的滴中，再次计时1min，时间到，再次收集胚胎放入第三个

0.25Su的滴中,计时1min（在此过程中会发现胚胎形态会越来越好）。

6、从培养箱中取出M16，用200μL的移液器分别吸取50μL M16做三个滴，从0.25Su的滴中挑出胚胎放入M16里清洗三遍。收集胚胎并计算存活胚胎数量，放入M16中进行培养和后续操作。

【注意事项】

1、冷冻时盖冻存管盖子时不能盖的太紧以免复苏时拧不开盖子而浪费时间；

2、冻存管开盖后，请立即将管口朝下，排掉可能浸入管中的液氮；

3、用移液器吹打液体时注意不要产生大量泡沫，且动作要尽可能快和轻柔。

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

Product	Item No.	specification	Application
0.75Su	72025	1mL	thawed embryos of mice
0.25Su	72026	1mL	thawed embryos of mice

Composition

Sucrose, Dulbecco’ s Phosphate-Buffer Saline(D-PBS).

Uses and Principles

This reagent is mainly used for warming embryos of mouse embryo cryopreservation, applicable to all periods from oocyte to blastocyst, generally need to be used with KOSM or M16.

Storage conditions and expiration date

1. Storage Temperature: 4℃,Protect from light.
2. Expiration date: 6 months. Discard unused reagents after opening.

Methods

1. 0.25Su, 0.75Su and M16 were pre-warmed in incubator for more than one and a half hours for embryo thawing, and M16 dishes covered with mineral oil were prepared in advance.
2. Maintain the cryovials at room temperature for 30 seconds.
3. Pipette 900μL of 0.75Su into the cryovials, and gently blow the bottom of the cryotubel with a pipette to observe whether the liquid at the bottom is thawed. After the liquid is completely thawed, transfer the sucrose solution in the cryovials to a 35 mm dish, and then pipette 400-500μL of 0.75Su into the cryovials repeat the above operation.
4. Remove 0.25Su from the incubator and make three 50uL drops on a 35mm dish.
5. Place the dish under the microscope, collect the embryos in the 0.75Su drop, and transfer them into the first drop of 0.25Su for 1min, wash them through the other two drops of 0.25Su every 1 min. During this process,you should see that the thawed

oocytes/embryos slowly swell up due to rehydration.

6. 50 μL M16 of three drops was pipetted into a dish, embryos were collected and washed three times in M16, and finally the number of surviving embryos was counted and placed in the mineral oil covered M16.

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. Unscrew the cryovials and immediately turn over the mouth of the tubeand drain the liquid nitrogen that may have immersed in the tube.

3. Be as gentle as possible when blowing the liquid with the pipette which prevent to appeare large amounts of foam.