精子冷冻保护剂使用说明书 （小鼠专用）

产品	货号	规格	用途
g-CPA	72022	1mL	小鼠精子冷冻保护剂

【主要组成成分】

棉籽糖、脱脂奶粉、L-谷氨酰胺等

【用途原理】

本试剂主要用于小鼠的精子冷冻保种，一般需要配合精子冷冻麦管、封口仪、HTF等使用。冷冻保护剂的主要作用就是，减少冷冻和解冻过程中细胞的渗透性损伤和减少冰晶的形成，尽最大可能减轻冻融过程对精子的损伤，提高冻融后精子的存活率和运动能力。

【储存条件及有效期】

1、储存条件：4-25℃，避光保存

2、有效期：6 个月，开口后未使用的试剂，请及时用封口膜密封保存

【冷冻小鼠要求】

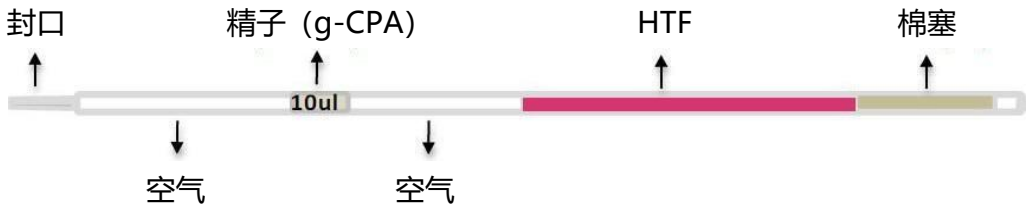
雄鼠，建议3-6月龄，繁殖笼的雄鼠使用前至少单放3天，非繁殖笼的库存雄鼠，实验前先试配后至少单放一周

【使用方法】

1、精子冷冻皿的制作：取两个35mm培养皿，从培养箱中拿出已平衡半个小时以上的g-CPA试剂，在每个培养皿中分别做一个120μL的滴，一个用于清洗，一个120μL的滴用于悬浮精子，其中悬浮精子的培养皿需要用矿物油完全覆盖放于37℃热台上。

2、精子采集：脱颈椎法迅速处死雄鼠，快速无菌分离双侧附睾尾，先用滤纸轻柔去除附睾尾上的血液和毛发。再将附睾尾放于盛有120μL g-CPA 滴的培养皿中（无矿物油覆盖），置于体视显微镜下，用显微镊和显微剪将脂肪组织去除。将两侧附睾尾转移至另一个120μL g-CPA 微滴中，用显微剪在每侧附睾尾上剪出5~6个切口，切口剪好后，及时的将培养皿在37℃热台上放置3min，每分钟需晃动一次g-CPA悬浮滴，保证精子充分且均匀的分布在液滴中。

3、精子冷冻：3min后，在热台上将精子悬浮液分成10个10μL的小滴（小滴可做在皿盖上）。取10根麦管，小心地在每个麦管中吸入100μL左右的HTF, 15 mm左右的空气柱，10μL的精子悬液然后吸空气到顶。制作完10根麦管，统一使用封口仪封口，再将麦管放在事先在液氮上的漂浮上，在低温蒸汽中预冷10min，10min后将麦管投入液氮中，冷冻结束。



冷冻麦管示意图

【注意事项】

1、试剂使用前请提前预热30min，该试剂不能放在-20℃中，会造成成分析出，无法使用。

2、精子冷冻时，在37℃热台上放置3min，每隔1min要同向晃动一次，使释放的精液均匀分布在液滴中。

3、封口时需需确认封口质量，避免漏管，若复苏时发现漏管，则不能水浴，直接放置培养箱隔板10min即可。

4、精子冷冻适用于绝大部分品系，个别品系会存在复苏效率低的情况。

5、如需复苏精子并得到胚胎移植，还需涉及c-TYH、HTF试剂。

6、精子冷冻前，应预先在有棉塞一侧的麦管外壁，标注小鼠的冷冻信息。

7、使用液氮时，请注意安全。

Cryoprotective agent (Mouse-specific)

Product	Item No.	specification	Application
g-CPA	72022	1 mL	Sperm cryoprotectant solution in mice

Composition

Raffinose, skimmed milk powder, L-glutamine, etc.

Uses and Principles

This reagent is mainly used for sperm cryopreservation of mice, It is to be used with sperm straws, sealers, HTF, etc. The role of cryoprotective agent is to improve the motility and survival rate of sperm after frozen-thawed sperm. Reduces intracellular damage caused by change in osmolarity.

Storage conditions and expiration date

1. Storage: 4℃-25℃, Protect from light.

2. Expiration date: 6 months. If the reagent is not used after opening, please seal it with sealing film in time.

Mouse

Male mouse 3-4 month of age, male mice in breeding colonies should be left alone for at least 3 days before use, and male mice in no- breeding colonies should be left alone for at least one week after trial mating before the experiment.

Methods

1. Preparation of dishes: g-CPA was prepared and equilibrated for at least 30 min at 37°.A 35mm culture dish with a 120μL g-CPA drop (for washing the epididymis) , another 120μL drop of g-CPA covered with oil was placed on the heating plate for sperm suspension.

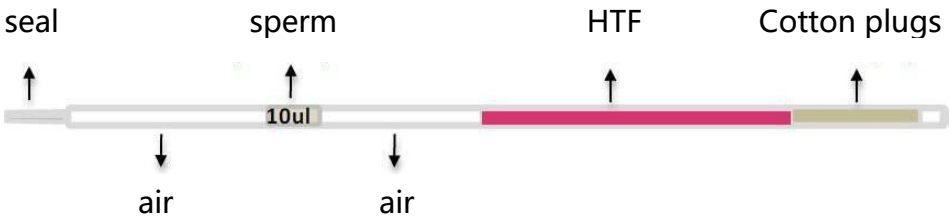
2. Harvesting vas deferens and cauda epididymis: Humanely euthanize male mice by cervical dislocation,Cut the vas deferens and gently pull out the vas deferens, removing most of the fat , hair and blood vessels.

3. Cut off the fat tissue just below the cauda epididymis and place the vas deferens and

cauda epididymis into the 35mm dish containing 120μL g-CPA (for washing the epididymis) .

4. Transfer cauda epididymis into another g-CPA drop（for release the sperm）,use spring scissors to make several cuts for all cauda epididymis to release the sperm, Place the dish on 37℃ hot plate for 3 min. gently swirled every min for 20 sec to help the sperm disperse from the tissue.

5. Cryopreserving sperm: the sperm suspension was divided into 10 aliquots of 10μL after 3 min, Take 10 straws, carefully aspirate about 100 μL of HTF, two 15mm of air column are spaced between 10 μL of sperm suspension , and seal the open end of the sperm straws. Place 10 sealed straws into the freezing canister, and float them on top of the LN_2 in the cooling chamber for 10 min. After 10 min , immerse the freezing container filled with liquid N_2.



Preparation and loading of the straw for sperm cryopreservation

Cautions:

1. The cryoprotective agent equilibrated for at least 30 min at 37℃ before use, the reagent cannot be placed in -20℃, it will cause into ingredients separate out and cannot be used.

2. When releasing the sperm, place the g-CPA drop on a hot plate at 37 ℃ for 3 min and swirl it in the same direction every 1 min so that the released sperm is evenly distributed in the drop.

3. When sealing sperm straws,we should be confirmed that the straws was sealed to avoid the spill of sperm , If the sperm is spilled during resuscitation from the straws, Water bath heating is prohibited and place it directly in the incubator for 10 minutes.

4. The cryoprotective agent is suitable for most strains, individual strains may have low recovery efficiency.

5. The c-TYH, HTF reagents has been required when the customer wants to transfer the embryos .

6. Before sperm cryopreservation,Mouse strain information pre-marked on the outer wall of sperm straws.

7. Ensure safety when using LN_2.