

体外受精液使用说明书（小鼠专用）

产品	货号	规格	用途
HTF	72002	2mL	小鼠受精液-新鲜精子
HTF-A液	72102	2mL	小鼠受精液-冷冻精子

【主要组成成分】

胚胎移植用水、BSA、双抗，酚红等

【用途原理】

本试剂主要用于小鼠新鲜精子体外受精，复苏精子体外受精，一般需要配合 c-TYH、A试剂等使用；NOD 品系做体外受精时，HTF可作为精子获能液使用。

【储存条件及有效期】

- 1、保存：4℃，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，并于24h内使用完毕。

【小鼠要求】

雄鼠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。

雌鼠C57BL/6品系采用3.5-4周龄，BALB/CJ、NOD背景建议8周龄。

【使用方法-HTF】

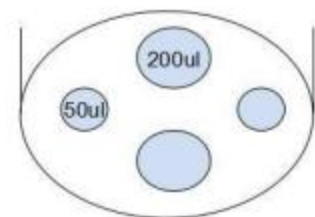
1、体外受精（新鲜精子，做受精皿用）

1.1 在35mm皿中做200 μ L的微滴2个（每滴放4-5个卵团）。

上覆矿物油，使用前需在培养箱中预平衡至少6h以上。

1.2 取出小鼠输卵管后，撕破输卵管膨大部，将释放的卵团放入到受精滴。

1.3 根据精子状态加入精子，此种培养皿当天不需换液，次日直接挑取2-cell培养。



HTF受精培养皿

2、体外受精（做获能皿用）

2.1 试剂使用前在培养箱中预平衡 30min。

2.2 脱颈椎法处死雄鼠后，打开腹腔迅速分离出两侧的附睾尾和输精管，将附睾尾放于滤纸上尽可能去除脂肪、血管和毛发。

2.3 用一次性 1mL注射器在附睾尾上开口（轻轻扎4-6下），挤出精液用显微镊让入精子皿微滴中，然后将含有雄鼠精液的精子皿放置于培养箱中获能 60min。

2.4 获能结束后，取出精子皿，从精子滴边缘吸取精子（因为有活力的精子都会游到滴的边缘），加到卵子受精滴中，根据精子的密度和活力情况，每滴建议加5-8 μ L左右的精子。

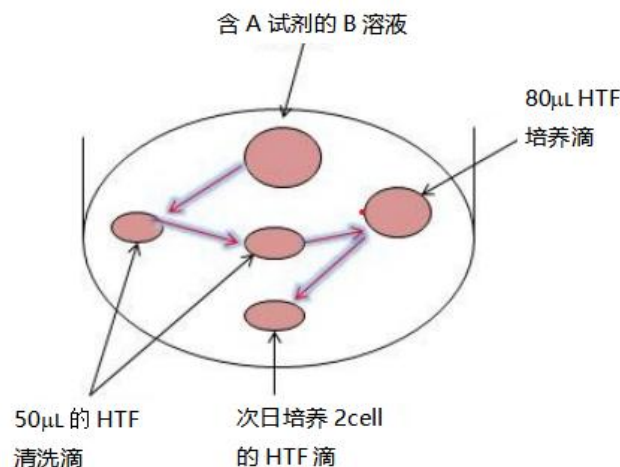
【使用方法-HTF-A液】

1、体外受精（复苏精子，做受精皿用）

1.1 拿取用EP管装的30.7mg的A粉末加入1mL HTF溶液，盖上盖子后，轻轻混匀，制备为“溶液A”备用。

1.2 吸取“溶液A” 10 μ L再加入990 μ L HTF溶液，混匀，此溶液称为“溶液B”，将用于制作受精滴。

1.3 在35mm的皿中先用“溶液B”做一个90 μ L的受精滴，然后用HTF做3个50 μ L的小滴和一个80 μ L的滴，其中两个小滴用于清洗，另一个小滴用于次日培养2cell，此外80 μ L的HTF培养滴，用于清洗后的培养。具体功能见下图。



1.4 然后把培养皿放入37 $^{\circ}$ C，5%二氧化碳培养箱中平衡30分钟后使用。一般情况下，一个受精皿对应1只供体雌鼠（约30~40枚卵子），每个受精滴需加入10 μ L获能后的复苏精子，加入精子4h后换液，清洗掉颗粒细胞及精子（50 μ L HTF），再放入80 μ L HTF中进行培养。

【注意事项】

- 1、卵子采集时时避免血液、毛发等进入到受精滴中。
- 2、在精子复苏使用HTF-A时，B溶液需要现配现用，建议不超过2小时，否则会影响受精率。
- 3、精子获能后，建议吸取最外圈精子使用。
- 4、HTF为安剖瓶装试剂，瓶子较薄，使用时注意安全。

In vitro fertilization medium (Mouse-specific)

Product	Item No.	specification	Application
HTF	72002	2mL	Mouse fertilization fluid-Fresh Sperm
HTF-A	72102	2mL	Mouse fertilization fluid-cryorecovered sperm

Composition

Water for embryo transfer, BSA, Penicillin-Streptomycin, Phenol Red.etc

Uses and Principles

The reagent is mainly used for in vitro fertilization of fresh sperm and frozen-thawed sperm in mice, which generally needs to be used with c-TYH. With NOD strains,HTF can be used as sperm capacitation medium.

Storage conditions and expiration date

1. Storage: 4°C, Protect from light.
2. Expiration date: 6 months. If the reagent is not used after opening, please seal it in time and use it within 24 hours.

Mouse

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.

Select female of C57BL/6 (3.5-4 week’s old)strains ,BALB/CJ and NOD strains were recommended at 8 weeks of age.

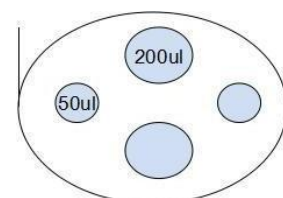
Methods

1. IVF- (fertilization dish for fresh sperm)

1.1 The 200μL drops were used for fertilization,transfer cumulus masses from all three femals to a single fertilization drop. Incubate the fertilization dishes at 37°C.

1.2 Collect oocyte-cumulus complexes by tearing the oviduct ampulla under a stereos -copic microscope in a drop of 200μL HTF.

1.3 2-cells will be picked directly the next day.



HTF medium

2. IVF- (HTF could be use as sperm capacitation medium for NOD strains)

2.1 A 35-mm culture dish with HTF covered with oil was prepared and equilibrated for at least 30 min in the incubator.

2.2 Collect tail of epididymis from a male killed via cervical dislocation or anesthetized, and place them on a filter paper. Remove the fat and blood from the epididymides.

2.3 A 1 mL syringe was used to gently punctured 4-6 times in the tail of the epididymis, Squeeze tail of the epididymis by the hands and help the sperm outflow.

Transfer of sperm clumps using microforceps for placement in HTF for 60 min in the incubator .

2.4 After sperm capacitation, pick up 10 μ L of sperm suspension from the edge of the drop was added to the HTF dishes containing oocytes. Depending on the density and viability of the sperm, it is recommended to add about 8 μ L of sperm.

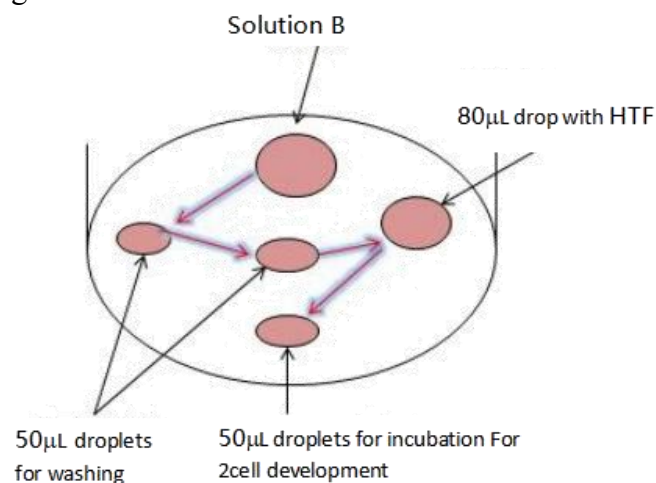
Usage - HTF-A

1. IVF-For preparation of cryorecovered sperm

1.1 Take 30.7 mg of A powder in an EP tube and add 1 mL of HTF solution, cover and mix gently to prepare "Solution A".

1.2 Pipette 10 μ L of "Solution A" into 990 μ L of HTF solution and mix well, this solution is called "Solution B" and will be used to make fertilized drops.

1.3 In a 35mm dish make a 90 μ L fertilization drop with "Solution B", then make three 50 μ L droplets and a 80 μ L drop with HTF, two 50 μ L droplets for washing and another 50 μ L droplet for culture for 2-cell culture, in addition, 80 μ L of HTF culture drop was used for the culture after washing. The specific functions are shown in the figure below.



1.4 The HTF dish was equilibrated in an incubator for 30 minutes before use. In general, one fertilization dish corresponds to one donor female mouse (about 30-40 eggs). Add 10 μ L of capacitated sperm to each fertilization drop(Solution B). After adding sperm for 4 hours, wash off granulosa cells and sperm, then culture in the 80 μ L drop of HTF.

Cautions

1. Avoid blood, fat and hair and tissue from entering capacitation drops during oocyte collection.
2. Solution B needs to be formulated when using, not to exceed 2 hours about time of use , otherwise it will affect the fertilization rate.
3. After sperm capacitation, it is recommended that pick up sperm suspension from the edge of the dropwas added to the HTF dishes.
4. HTF is packed in ampoule, the bottle is thin, please ensure safety when using it.