mC&I Device Instructions (Catalog #60020)

PRODUCT INFORMATION

Catalog #: 60020 (EtO Processed)
Unit Quantity: 10 devices per box (sold in box quantities only) 1 Box = 1 Unit

FOR TECHNICAL SUPPORT

Please Call: +1(859)317-9213 or Email: info@paratechs.com

IMPORTANT! Before using the mC&I device, please read instructions carefully.

Intended Use

This device is used for non-surgical transcervical transfer of mouse sperm or liquids for the study of uterine physiology into female recipient mice. For research purposes only. **Not intended for human or animal diagnostic or therapeutic uses.**

Other Use

The mC&I 60020 device can also be used for pathogen transfer into recipient female mice. If you are interested in the *Chlamydia* protocol, please send your request to info@paratechs.com.

Handling

Devices are single use only. Discard after use.

Non-Surgical Artificial Insemination Protocol for use with the C&I Device for Mice

This protocol was adapted from the non-surgical artificial insemination protocol for mice using the NSET device for CD1 female recipients and fresh sperm[1]. The transfer procedure video can be seen on the ParaTechs website (mNSET Quick Procedure). Please familiarize yourself with the mouse handling technique for the mNSET procedure prior to attempting this AI protocol.

Animals:

Female mice (> 8 weeks old)
Fertile male mice as sperm donors (preferably fertile males mated 4-7 days prior to AI)
Male vasectomized mice (VASEX= vasectomized male)

Equipment:

mC&I device (ParaTechs #60020)
P-200 pipette
1cc syringes, 26-gauge needles (for injections)
Scissors, forceps
Tissue culture dishes (60mm)
CO_2 incubator, 37˚
Hemocytometer (optional)
Dissecting microscope
Wire-topped cage
Kimwipes

[1] **Stone BJ, Steele KH, Fath-Goodin A** (2015) A rapid and effective nonsurgical artificial insemination protocol using the NSET™ device for sperm transfer in mice without anesthesia. Transgenic Res **24(4)**:775-81.

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Reagents:
PMSG; Pregnant Mare Serum Gonadotropin (Prospec # hor-272)
hCG; human Chorionic Gonadotropin (Prospec # hor-250)
CARD Fertiup Preincubation Medium: PM (Cosmo Bio USA Cat # KYD-002-EX) [or Human tubal fluid medium (HTF) (Irvine Scientific # 90126) with 4mg/ml BSA (Sigma # A3311-10G), filter sterilized] equilibrated in 5% CO_2 37°C incubator for 30 min.
Paraffin oil (Sigma-Aldrich # 18512-1L)

Procedure:

1. **Hormone Injection to induce ovulation on Days 1 and 3:**
   Note: The timing of injections, sperm transfer, and light cycle relative to each other are very important. The ParaTechs vivarium maintains a 12-hour light cycle. The times specified for all procedures in this protocol are based on a 12-hour light cycle (7:00 am to 7:00pm). You may need to adjust your injection times accordingly. Please pay special attention to this step.

   a. Day 1: PMSG injection (2.5IU) intraperitoneal at 6:30 pm.

   b. Day 3: hCG injection (2.5IU) intraperitoneal at 6:00 pm.

2. **Sperm Transfer Day 4:**
   Note: Prepare sperm collection dish with a 500µl drop of PM medium under Paraffin oil and equilibrate at least 30 minutes at 37°C with 5% CO_2 prior to sperm collection.
   a. At 8:00 am: Euthanize males with CO_2 1 hour prior to expected sperm transfer.
      - Moving quickly, dissect the cauda epididymides from the mouse. Transfer epididymides to a Kimwipe and remove fat and blood.
      - Place 2 cauda into the 500µl drop of pre-gassed PM under Paraffin oil. Cut the cauda epididymides making 6 incisions using small scissors or a 26-gauge needle.
      - Gently shake the dish and allow sperm to swim out for 2-3 minutes.
      - Remove all tissues.
      - Incubate sperm at 37°C with 5% CO_2 to capacitate for 45 minutes to 1 hour.

   b. Optional: Measure the sperm count on a hemocytometer and note motility and quality of sperm sample (@15 minutes prior to transfer). For counting, sperm can be diluted in sperm collection medium. An initial dilution of 1:10 is suggested.

   c. Optional: Record the weight of the female recipient prior to insemination.

   d. At 9:00 am: Deliver sperm to the uterine horn.
      - Place the mC&I device onto a P-200 pipette that has been set to 40 µl.
      - Press pipette plunger to first stop, lower tip into media at the edge of the sperm sample and slowly load sperm into the device. A microscope can be used to visualize sperm loading. Avoid clumps. Set aside pipette. **Important Note: Paraffin oil transfer to the uterine horn must be avoided**; remove oil from the exterior of the catheter using a Kimwipe. If desired, the sperm sample for transfer can be removed from the dish with oil and transferred to a fresh dish to minimize oil. However, sperm loses motility rapidly without the correct temperature and pH, so keep the sample at 37°C with 5% CO_2 as much as possible.

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- Place the unanesthetized recipient female on the top of a cage with a wire rack, allowing the mouse to “grab” the cage bar surface. Grasp near the base of the tail using thumb and forefinger and angle the tail upward while stabilizing the animal. Note: This handling technique takes practice, but when done correctly the mouse will hold still for the duration of the procedure. Anesthesia may be used during training sessions or if a particularly aggressive mouse strain is chosen as the recipient without affecting pregnancy.

- Place small speculum into vagina.

- While holding the female mouse with one hand as described above, carefully pick up the pipette and insert the mC&I catheter tip into the speculum, through the cervix and into the uterus. Once the mC&I hub contacts the speculum, expel sperm by pressing the plunger to the first stop. Note: Avoid transfer of extra air to the uterine horn.
- Remove the mC&I device and speculum. No post-procedure monitoring is required.

e. Immediately pair the female with a VASEX male overnight. Note: Copulatory activity is required to obtain pups from this procedure but not for embryo fertilization. The vasectomized males should mate within a few hours.

f. Visually check for a copulation plug before the end of the day. Visual inspection and/or a blunt-end probe may be used to determine the presence of a vaginal plug.

3. **Dissolve Mating Pairs Day 5:**
a. Remove the female from the VASEX male cage. Visually check for a copulation plug again. This is best done as early as possible in the day (before 9:00 am) as a copulation plug may fall out. Note: some females without visible plugs may litter if copulatory activity occurred.

4. **Optional: Pregnancy check Day 11**
Weight gain (~2g) can be used to determine pregnancy status by around 11 days.

PARATECHS COROPORATION LIMITED WARRANTY

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Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product.

Patent Information: Non-Surgical Embryo Transfer Method and Apparatus, **United States Patent 9,615,903**. [PDF]

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