

**Procédure Normalisée de Fonctionnement**

<b>TITRE :</b> IMPLANTATION SOUS-CUTANÉE DE CELLULES TUMORALES HUMAINES CHEZ LA SOURIS IMMUNODÉFICIENTE	<b>NUMÉRO :</b> CHX-2
<b>DESTINATAIRES :</b> Personnel du Service des animaleries et usagers	<b>Version 1 :</b> 2007 <b>Version 5 :</b> 20.05.2016
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<b>BUT :</b> Décrire les étapes de l'implantation sous-cutanée de cellules tumorales chez la souris immunodéficientes	

**MATÉRIEL :**

- Female mice Crl: CD-1 *Foxn1<sup>nu</sup>* 28-42 days from Charles River laboratories
- (ATCC HTB-)
- Methylcellulose (SIGMA M-0512)
- Isofluran anesthetic gas machine
- Isofluran
- Anesthetic mask
- Induction cage
- Sterile syringe BD 1ml with 26G3/8 needle for subcutaneous injection (1/mouse)
- Proviodyne 1%
- Sterile cotton gauzes
- 70% alcohol
- 1% Virkon solution
- Sterile self piercing ear tags
- Sterile tags from National Band and Tag Co #1005-1 MONEL
- Sterile surgical fields
- Electric heating pad

**PROCÉDURES :**

1. All material, which may enter in contact with mice during procedures, must be sterilized with steam or disinfected with a 1% Virkon solution and washed with 70% alcohol.
2. The mouse (female Crl: CD-1 *Foxn1<sup>nu</sup>* 20-25g) is anesthetized in the induction cage with a combination of Isofluran (5% concentration) and medical oxygen (1L/minute).
3. When the mouse is asleep, the Isofluran concentration is diminished between 1.5 and 2.5% (oxygen rate stay the same).
4. The mouse is then weigh and identified with a sterile ear tag on the left ear.
5. The mouse is maintained anesthetize with the mask and put in left lateral decubitus on the heating pad previously covered with a sterile surgical field (heat the pad at low or med).

6. The exposed part of the mouse is disinfected with sterile gauze saturated with proviodine 1% solution or 70% alcohol.
7. The tumor cell suspension ( $2.5 \times 10^6$  cells/100 $\mu$ l in MEM + 1% methylcellulose) is «vortexed». Tumor cell suspension is kept at room temperature during all the procedure.
8. 100 $\mu$ l of the tumor cells suspension is sucked in the syringe.
9. The needle is then gently inserted subcutaneously in the right flank between the inferior paw and the last rib.
10. The tumor cell suspension is slowly injected.
11. A gentle pressure is made on the entering point of the needle (to prevent leakage of the tumor cell suspension) and the needle is quickly removed.
12. The mouse is taken off anesthesia and stays under observation until full recovery.

## **TUMOR FOLLOW-UP**

### **Measurement:**

- A digital caliper 2 decimals precision is used for tumor measurement.
- Tumor measures begin at day 4 post-implantation and then 2 times a week until treatment begins. After that, tumors are measured the day of each treatment and the day following treatment.
- Formula used to calculate tumor volume is  $(\text{length} \times \text{width}^2) \times 0.52$ .
- Mice are sacrificed and necropsy is performed when tumor volume reaches 1000mm<sup>3</sup>.

### **Treatment:**

- Treatment begins when tumor average volume reaches 150-200mm<sup>3</sup> (between 12 to 18 days post-implantation).
- Mice are randomized in groups where  $n=5$  to 8 mice.