

POST-COCAINE SENSITIZATION TISSUE COLLECTION

(TC V1.0)

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1. Purpose and Scope

Post-cocaine sensitization tissue collection of brain and body tissue for the purpose of high quality RNA or DNA extraction. Tissues collected: right hemisphere of the brain, left hemisphere dissections of: hippocampus, striatum, prefrontal cortex, liver, spleen, and tail, fecal boli collected from within the cecum, and cecum tissue.

2. Materials

- Liquid nitrogen in LN2 dewar $\frac{3}{4}$ full
- Microscope (Nikon SMZ800N)
- Decapitation scissors (Fine Science Tools, Item no. 14200-21)
- Surgical scissors (Fine Science Tools, Item no. 14058-09)
- Forceps (Fine Science Tools, Item no. 11052-10)
- Razor blade (Electron Microscopy Sciences, Item no. 71970)
- Nunc Cryotube Vials (Thermo Fischer Scientific, Item no. 363401)
- 2 dissecting fine angled tip knives (Fine Science Tools, Item no. 10056-12)
- Rongeurs (Fine Science Tools, Item no. 16020-14)
- RNase away (Sigma-Aldrich, Item no. 83931)
- Haemo-sol (Haemo-Sol International)
- Spray bottle with 70% ethanol solution

3. Definitions and Acronyms

4. Safety Key Points

- 4.1. Clean all tools with haemo-sol, RNase away, and 70% ethanol between each subject.
- 4.2. Verify the routine sharpening of decapitation scissors for swift and efficacious euthanasia, according to institutional IACUC guidelines.
- 4.3. For animal health and safety, only biometrics certified individuals are qualified to perform euthanasia.
- 4.4. Handling of Liquid nitrogen should be done in accordance with laboratory safety guidelines. Dispense LN2 into the dewar only while wearing a face shield and protecting

handwear. Removal of tubes from the dewar should be accomplished with a slotted spoon or long forceps.

5. Quality Key Points

- 5.1. Obtain the correct tissues in their appropriately marked vials, confirming mouse ID at each step.
- 5.2. Some tissues, particularly brain regions, are very “sticky” and can carry adjacent brain regions during collection. Pay marked attention to Striatum collection to minimize non-striatal carry-over, thus maximizing gene expression results.

6. Procedure

6.1. Subjects

6.1.1. Species: Mice

- 6.1.1.1. CSNA mouse colonies (E.g. strain, sex, date of birth), post weaning age.

6.2. Tissue Collection

- 6.2.1. Prior to tissue collection, Nunc brand cryotube vials should be labeled with subject ID and type of tissue, with seven tubes per subject. Mix haemo-sol according to manufacturer’s instruction.
- 6.2.2. Sanitize the work area with 70% ethanol spray.
- 6.2.3. Wearing gloves, restrain subject, note any mouse behavior or visual abnormalities on an electronic collection sheet and use decapitation scissors to perform a live decapitation.
- 6.2.4. Quickly, use surgical scissors to remove brain from skull. Use a new razor blade to cut along the longitudinal fissure through the corpus callosum of the brain, separating left from right hemispheres.
- 6.2.5. Using forceps, isolate right hemisphere of brain into properly labeled tube. Place into liquid nitrogen.
- 6.2.6. Using a microscope (70x-90x, adjusted for personal use) isolate hippocampus, then striatum, and then prefrontal cortex from left hemisphere of brain and place into respective tubes and cap tightly. Carefully place tubes into liquid nitrogen filled dewar.
- 6.2.7. Discard leftover brain regions and skull.
- 6.2.8. Make an incision through the ventral lower abdominal skin, and pull the skin away, incising along the lateral body edges revealing the peritoneum. Incise the abdominal wall to expose the body organs. Note the visual appearance of the viscera documenting any abnormalities (e.g. enlarged spleen, fluid pockets, color changes, missing organs or unexpected masses) in an electronic tracking sheet containing all subject and collection information. Isolate the left lobe of the liver and put into labeled tube. Remove spleen. Cut off whole tail at base and section into thirds, placing all three into one cryo-tube. Place tubes into liquid nitrogen.
- 6.2.9. Carefully, remove tubes from liquid nitrogen and store at -80 degrees Celsius until analysis.