School of Biomedical Sciences



**Standard Operating Procedures**

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| **Title** | Golgi-Cox Solution Use and Staining Methodology | |
| **Date** | 11/04/2022 | |
| **Equipment** | **Make: NA Model: NA** | |
| **Location** | **Bld: 65 Room: 210** | |
| **Equipment**  **Custodian** | **Contact: Dr Darryl Whitehead** | **Expert user: Miss Erica Mu** |
| **Task** | This task is performed on both human and animal specimens, in both a teaching and research environments for validating the presence of neuron structure and pathways. | | |
| **Pre start checks** | * Ensure appropriate Risk Assessment has been read online (RiskWare ID: 1626) * Validate operation and availability of fume cupboards in facility * Order in appropriate Chemwaste waste containers prior to use * Validate histochemical ingredient status * **Ensure appropriate training prior to use and organise supervision during use** * Plan and book Histology Facility at low traffic periods | | |
| **Safety considerations** | **Personal Protective Equipment (PPE):** safety glasses (RA), lab coat, gloves (RA) and fully enclosed shoes,  **General precautions**:   * Long hair must be tied back; loose objects from head/neck/ sleeve area must be covered by lab coat. * **Chemical preparation of stain MUST be performed within normal working hours (8am-6pm) under direct supervision of an authorised person** * Avoid exposure to all chemicals during solution preparation and staining * Always alert your supervisor if you or someone around you if feeling sick/ faint * Do not do this procedure in a position where you are likely to be bumped into * Ensure adequate low lighting when required for the procedure * Avoid exposure to all chemicals during solution preparation and staining * Chemicals are not to leave the Histology Facility unless approved prior * Ensure appropriate knowledge on how to use heated stirrer. Avoid contact with hot plate as it may cause burns.   **Emergency Procedures:**  In the event of an emergency, inform Histology Facility staff, WHSO, and/or security ext 53333 (When calling Security inform of chemical exposure).  All incidents should be reported to the Facility Staff and Manager, Ext 51929, Safety Coordinator, Ext 53221, and/or Security 53333.  All injuries must be reported to SBMS HSW Management, Ext 53221 or 51269, Building Management, Ext 53105. | | |
| **Procedure** | **Golgi-Cox Histochemical Preparation**  **Note: Must be prepared in a fume hood under low light conditions. Highly TOXIC. Must be prepared according to the following instructions. Label solution with correct Chemwatch chemical labels at all times.**  Makes ~500mL of solution (~30mL solution per brain sample)  Solution A: 5% Potassium dichromate solution (make in 250mL beaker)  5g of potassium dichromate dissolved in 100mL of distilled H2O.  Solution B: 5% Mercuric chloride solution (make in 250mL beaker)  5g of mercuric chloride dissolved in 100mL of distilled H2O.Use a magnetic stirrer and a hot plate (low-medium heat) to dissolve crystals.  Solution C: 5% Potassium chromate solution (make in 500mL beaker)  4g of potassium chromate dissolved in 80mL of distilled H2O.  Method:  1. Further dilute Solution C by adding 200mL of distilled H2O.  2. Mix Solution A with Solution B slowly thus creating Solution AB.  3. Pour Solution AB slowly into Solution C while stirring on a hot plate (low-medium heat).  4. Stir on hot plate until dark orange to red in colour. Solution will be cloudy when completed.  5. Pour completed solution into a plastic Golgi-Cox solution container, validate the exterior of the bottle and cap are clean and residue-free.  6. Label the container appropriately and place in the 'dark' for 5 days (ie. Lockable chemical cupboard as specified by Histology facility staff). Check DAILY for any signs of leakage or oxidation.  5. Following 5 days, filter fresh Golgi Cox solution into a new Golgi-Cox solution container in the fume hood and store and label appropriately. This is the working solution. This is stable for approximately 14 days.  **Golgi-Cox Histochemical Staining**  1. Pour 30mL of filtered Golgi-Cox solution into a sealable pre-approved specimen container.  2. Remove freshly excised brain (can keep whole or halve hemispheres) and place sample into specimen container.  3. Ensure tubes are labelled correctly.  4. Incubate at 37°C. Incubation times may vary and should be optimised for the tissue of interest eg. Incubate for 6 days (for 7-8wo male Wistar rat amygdala).  Following the staining period, discard all waste solution and remaining stock solution in appropriate chemical waste container.  **Golgi-Cox Histochemical Post-processing**  **Note: Must be prepared in a fume hood under low light conditions. Highly TOXIC. Must be prepared according to the following instructions. Label solution with correct Chemwatch chemical labels at all times.**  1. Cut stained specimen on vibratome (Refer to Histology lab SOP – Operating the vibratome), and place slices in well plates containing 30% sucrose in 0.1M PBS or distilled water.  2. Dehydrate slices in 50% ethanol for 5 minutes.  3. Place in 0.1M ammonia solution for 30 minutes in the dark. Cover with alfoil.  4. Rinse twice with distilled water for 5 minutes each.  5. Place in Fujifilm or photo fixer solution for 30 minutes in the dark. Cover with alfoil.  6. Rinse twice with distilled water for 2 minutes each.  7. Dehydrate through series of ethanol solutions: 70%, 90%, 95%, 100%, 100% and 100% ethanol for 5 minutes each.  8. Place in CXA solution (1:1:1 chloroform: xylene: alcohol) for 10 minutes. Note: If solution becomes cloudy, there is water contamination, dispose of waste solution in appropriate waste container. Make up fresh solution.  9. Clear in two changes of xylene, 5 mins each.  10. Mount on slides with DepeX, and coverslip. Leave to dry in the fumehood in the dark overnight, or until slides have completely hardened. Cover with alfoil to prevent light exposure.  11. When storing slides, wrap slide box with alfoil to prevent light exposure and oxidation and store at room temperature. | | |
| **Legislative requirements** | * AS 2243. Safety in Laboratories. | | |

[[1]](#endnote-1)

1. Date of issue: 11/04/2022

   Next review: 11/04/2025 [↑](#endnote-ref-1)