FD Rapid GolgiStain™ Kit

A complete Golgi-Cox staining system for the study of the morphology of neurons and glia

User Manual

PK 401/401A, Version 2014-02

FOR *IN VITRO* RESEARCH USE ONLY
not for diagnostic or other uses

FD NEUROTECHNOLOGIES, INC.

Quality & Excellence Since 1996

FD NeuroTechnologies
Consulting & Services, Inc.

Contents

I. Introduction

Golgi-Cox impregnation[2, 5] has been one of the most effective techniques for studying both the normal and abnormal morphology of neurons as well as glia. Using the Golgi technique, subtle morphological alterations in neuronal dendrites and dendritic spines have been discovered in the brains of animals treated with drugs as well as in the postmortem brains of patients with neurological diseases[1, 3]. However, the reliability and time-consuming process of Golgi staining have been major obstacles to the widespread application of this technique.

FD Rapid GolgiStain™ kit is designed based on the principle of the methods described by Ramón-Moliner[5] and Glaser and Van der Loos[4]. This kit has not only dramatically improved and simplified the Golgi-Cox technique but has also proven to be extremely reliable and sensitive for demonstrating morphological details of neurons and glia, especially dendritic spines. The FD Rapid GolgiStain™ kit has been tested extensively on the brains from several species of animals as well as on the specimens of postmortem human brains (for photo samples and references using this kit, please visit our web site at www.fdneurotech.com).

II. Kit Contents

Store at room temperature

	PK401A	PK401
Solution A	125 ml	250 ml
Solution B	125 ml	250 ml
Solution C	125 ml x 2	250 ml x 2
Solution D	125 ml	250 ml
Solution E	125 ml	250 ml
Glass specimen retriever	2	2
Natural hair paintbrush	2	2
Dropping bottle	1	1
Plastic forceps	1	1
User manual	1	1

III. Materials Required but Not Included

1. Double distilled or Milli-Q water
2. Plastic/glass tubes or vials
3. Histological supplies and equipment:
    - Gelatin-coated microscope slides (Cat. #PO101)
    - Coverslips
    - Staining jars
    - Ethanol
    - Xylene
    - Permount®
    - A light microscope.

Permount® is a registered trademark of Fisher Scientific.

IV. Safety and Handling Precautions*

1. FD Rapid GolgiStain™ kit is made for *in vitro* research use only and not for drug, diagnostic or other uses.
2. The kit contains reagents that are toxic and harmful in contact with skin or by inhalation and may be fatal if ingested. Do not pipette by mouth. Avoid inhalation and contact with skin and eyes. In case of contact, wash immediately with generous amounts of water and seek medical advice. If swallowed, wash out mouth with water and immediately call a physician.
3. Perform experiment under a chemical hood. **Wear suitable protective clothing, gloves and eye/face protection while handling kit reagents.** Wash hands thoroughly after performing the experiment.

*Material safety data sheet (MSDS) is available at www.fdneurotech.com.

V. Tissue Preparation

The following instructions must be read before using this kit.

- All containers (plastic preferred) to be used should be cleansed and rinsed with distilled water.
- Do not use metal implements whenever Solutions A and B are present.
- Keep containers tightly closed at all times.
- Tissues treated with Solutions A and B, including sections should be protected from light whenever possible.

- The following procedure should be performed at room temperature unless specifically indicated.

1. Experimental animals should be deeply anesthetized before killing. The animal brain (or postmortem human specimens) should be removed from the skull as quickly as possible but handled carefully to avoid damage or pressing of the tissue.

   Note

   - *Do not perfuse animals unless absolutely necessary. If you do need to perfuse animals (with 4% paraformaldehyde for less than 5 minutes), tissue must not be postfixed.*
   - *Large brain specimens, including rat brains should be sliced with a sharp blade into blocks of approximately 10 mm thickness.* ***Important!***

2. Rinse tissue quickly in double distilled or Milli-Q water to remove blood from the surface.

3. Immerse tissue in the impregnation solution, made by mixing equal volumes of Solutions A and B, and store at room temperature for 2 weeks in the dark*. Replace the impregnation solution after the first 6 hours of immersion or on the next day.

** A 2-week impregnation time is satisfactory in most cases. However, variations in type and actual size of tissue may require a shorter or longer duration of impregnation to obtain the best results. The optimal time should be obtained by trial for each type of tissue, but 3 weeks should be sufficient for most tissues. Note that prolonging the impregnation time may increase background staining.*

Note

- *The mixture of Solution A and B should be prepared at least 24 hours prior to use and left unstirred.*
- *It is important to use the top part of solution that is free of precipitate.*
- *The impregnation solution may be stored at room temperature for up to 1 month in the dark before use.*
- *Use at least 5 ml of the impregnation solution for each cubic cm of the tissue to be studied. Note that use a lesser volume of impregnation solution may decrease the sensitivity and reliability of staining.*
- *For the best results, gently swirl (do not shake!) the tissue container side-to-side for a few seconds twice a week during the period of impregnation.*

Warning:

Solutions A and B (containing mercuric chloride, potassium dichromate and potassium chromate) are toxic in contact with skin and may be fatal if swallowed. The experiment should be performed under a chemical hood. Wear suitable protective clothing, gloves and eye/face protection while handling the reagents. DO NOT POUR THE WASTE OF SOLUTIONS A AND B INTO THE SINK. Collect the waste of these solutions in a bottle and call your safety office or a licensed professional waste disposal service to dispose of this material.

4. Transfer tissue into Solution C and store at room temperature in the dark for at least 72 hours (up to 1 week). Replace the solution at least once after the first 24 hours of immersion or on the next day.

5. 100 to 200 µm sections can be best cut on a cryostat at -20°C to -22°C (read carefully the information on page 10 before sectioning). Other types of microtomes, including sliding microtome and vibratome, can also be used (for tips, cf. pages 10 & 11). Each section should then be transferred with a glass specimen retriever (provided) and mounted on gelatin-coated microscope slides (Cat. #PO101) with Solution C (a dropping bottle is provided for easy dropping of Solution C onto the slides). Excess solution left on slide should be suctioned with a Pasteur pipette and then absorbed with a strip of filter paper (solution on slide must be wiped away as much as possible or sections will fall off slides). Allow sections to dry naturally at room temperature (do not use a fan or hot plate). For the best results, sections should be processed as soon as possible, but may be stored in a slide box at room temperature in the dark for up to 3 days.

Note

- *To prevent tissue from ice crystal damage and to preserve the best possible cell morphology, tissue should be frozen rapidly before sectioning with a cryostat. For example, tissue may be rapidly frozen as described below: place tissue in a plastic spoon and slowly dip into iso-pentane pre-cooled with dry ice (for the best results, temperature should be kept below -70°C and the dipping should take about 1 min, the slower the better). After the tissue is completely immersed in iso-pentane, keep it in iso-pentane for a few seconds and then place it on dry*

ice for another minute to ensure that the tissue is well frozen. Do not let tissue thaw before sections are cut.

- *The types of cryostat may vary, but all types should be able to cut thick sections (e.g. 100 µm). Contact FD NeuroTechnologies for technical assistance.*
- *If the cryostat has only one temperature control, set the cryostat temperature to -22°C at least 4 hours before cutting. If the cryostat has 2 temperature settings, set the chamber temperature 1 degree lower (colder) than that of the specimen head. Please note that -22°C is satisfactory in most cases. However, variations in type of cryostat and tissue may require a higher or lower chamber temperature in order to cut sections smoothly and without shattering.*
- *The following tips should be helpful for sectioning with a cryostat: 1) Mount tissue on specimen disc (also called chuck or stage) with distilled water, but make sure that the tissue is not thawed (may be done on dry ice). Tissue may also be mounted with any type of tissue freezing medium, including OCT, but avoid cutting through the medium. Do not embed the tissue in OCT. If the tissue has to be embedded for cutting, use TFM (TBS, Durham, NC, USA, Cat. #TFM-5) instead; 2) After the tissue is mounted on specimen disc, place the tissue on dry ice for 10 minutes, and then immediately install the specimen disc together with the tissue on a cryostat and wait 5 minutes before cutting; 3) Cut a few sections (do not use the anti-roll plate). If the tissue is too cold, e.g. sections show cracks which are parallel to the blade, wait a few more minutes before the next trial. Otherwise continue to cut.*
- *The impregnated brain may also be embedded in agarose or gelatin and cut with a vibratome. However, for collecting sections (filling the chamber), Solution C must be used.*

Otherwise, sections may crack upon drying.

- *To cut the impregnated tissue with a sliding microtome, both the stage and knife (or blade) need to be maintained at low temperature (below 0°C). It is also important that sections should be mounted with solution C as described in the User Manual.*

VI. Staining Procedure

Do not let sections dry out between any steps during the staining and before coverslipping.

1. Rinse sections in double distilled or Milli-Q water 2 times, 4 minutes each.

2. Place sections in a mixture consisting of 1 part **Solution D**, 1 part **Solution E** and 2 parts double distilled or Milli-Q water for 10 minutes.

e.g. **Solution D**	10 ml
Solution E	10 ml
Double distilled water	20 ml

Note

- *The working solution should be prepared just before use and may be used for up to 100 sections (e.g. mouse brain) per 100 ml, depending on the size of sections.*
- *The bottle and staining jar containing the working solution must be covered to prevent vaporization of the reagent.*

- *For the best results, the working solution should be stirred frequently during incubation.*

3. Rinse sections in double distilled or Milli-Q water 2 times, 4 minutes each (distilled water should be renewed frequently).

4. Counterstain sections with cresyl violet or thionin (optional step).

Note

- *For counterstaining, step 3 must be prolonged, e.g. instead of 2 times and 4 minutes each, wash 4 times and 5 minutes each or longer.*

5. Dehydrate sections in 50%, 75% and 95% ethanol, 4 minutes each (do not skip any step).

6. Dehydrate sections in absolute ethanol, 4 times, 4 minutes each (do not prolong).

7. Clear in xylene, 3 times, 4 minutes each, and coverslip with Permount®.

Note

- *Sections may be temporally stored in xylene for a few hours before coverslipping.*
- *For the best results, use undiluted Permount®.*
- *Golgi-stained sections should be protected from light whenever possible.*

VII. References

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