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 2019-01

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

FD NeuroTechnologies, Inc.
Quality & Excellence Since 1996

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I. Introduction

The Golgi-Cox method (1987) has been one of the most effective techniques for studying the morphology of neuronal dendrites and dendritic spines. However, the reliability and time-consuming process of Golgi-Cox staining have been major obstacles to the widespread application of this technique. To overcome these shortcomings and to promote this invaluable technique, we have developed the FD Rapid GolgiStain™ Kit based on the principle of the methods described by Ramón-Moliner (1970) and Glaser and Van der Loos (1981).

This kit is reliable for visualizing morphological details of neurons, especially dendrites and dendritic spines. The kit is suitable for the analysis of various parameters of dendritic morphology—such as dendritic length and branching pattern and dendritic spine number, shape, and size—in different areas of both animal and postmortem human brains (Beggs, et al., 2003; An et. al., 2008; Milatovic et al., 2010; Koyama, 2013; Koyama et. al., 2015; Bicanic et al., 2017; Gstrein et al., 2018; for more references using this kit, visit www.fdneurotech.com). The kit can be used for processing large or small quantities of brain samples or sections all at once or over a period of time. A 40-min instructional video for tissue freezing, cryosectioning, and staining is provided (see www.fdneurotech.com).

The FD Rapid GolgiStain™ Kit should be used with fresh or shortly fixed brain tissue. Using with previously formalin-fixed or fresh-frozen brains is not recommended since satisfactory results with these types of samples are not typically obtained. Additionally, the kit does not work on any tissue that has already been cut, either free-floating sections or sections mounted on microscope slides (for more information, see Du, 2019, *Current Protocols in Neuroscience*, *88*, e69. doi: 10.1002/cpns.69).

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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
   - Eukitt® Quick-Hardening Mounting medium
   - Cryostat or vibratome
   - Upright bright-field microscope

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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

Please read the following instructions before using this kit.

See also an instructional video for tissue freezing and cryosectioning at www.fdneurotech.com or Current Protocols in Neuroscience, 88, e69. doi: 10.1002/cpns.69.

- 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.

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- Tissues, including sections treated with Solutions A and B, should be protected from light whenever possible.
- The following procedure should be performed at room temperature unless specifically indicated.

1. Prepare impregnation solution (Solution A/B) by mixing equal volumes of Solutions A and B at least 24 hours prior to use and leave unstirred.

   The impregnation solution should be stored at room temperature in the dark before use and used within 4 weeks.

   CAUTION: Solutions A and B (which contain mercuric chlride, potassium dichromate, and potassium chromate) are toxic if they come into contact with skin and may be fatal if swallowed. Do not breathe vapor or fumes from these solutions.

   CAUTION: *Do not pour the waste of Solutions A and B into the sink. Collect waste in a waste container, and contact the local safety office or a licensed professional waste disposal service to dispose of this material.*

2. Deeply anesthetize experimental animal with IACUC-approved methods before sacrificing.

   Do not perfuse animals unless absolutely necessary. If perfusion is necessary, tissue must NOT be postfixed (see step 3 for more details).

3. *Optional:* Perfuse animal with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) according to the following suggested protocol (e.g., for both Golgi-Cox impregnation and immunostaining).

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a. Perfuse animal with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 4 min.
b. Remove brain from skull immediately after perfsion.
c. Divide brain (e.g., mouse or rat brains) into 2 hemispheres or 5-mm thick blocks.
d. For Golgi-Cox staining, continue to step 5. DO NOT POSTFIX TISSUE!
e. For immunostaining, continue to process tissue according to desired protocol.

4. Remove animal brain (or postmortem human brain tissue) from the skull as quickly as possible. Be sure to handle carefully to avoid damaging or pressing the tissue.

   Large brain specimens, including rat brains, should be divided with a sharp blade into blocks of approximately 5 to 10 mm thickness. For example, a rat brain may be divided either sagittally into 2 hemispheres or coronally into 2 to 3 blocks depending on the regions of interest.

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

6. Immerse tissue in impregnation solution (Solution A/B; from step 1), and store at room temperature for 2 weeks in the dark. Replace impregnation solution after the first 6 hr of immersion or on the next day. Gently swirl (do not shake!) the tissue container side to side for a few seconds at least twice a week during the impregnation period.

   Use at least 5 ml impregnation solution for each cm3 of tissue. However, it is strongly recommended to use 1.5

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times the minimum amount of impregnation solution (e.g., for a mouse brain, instead of using 5 ml, use 7.5 ml). Note that using a lower volume of impregnation solution may decrease the intensity and reliability of staining. The impregnation solution should be prepared at least 24 hours prior to use and left unstirred. It is important to use the top part of the solution that is free of precipitate.

In most cases, 2 weeks of impregnation is satisfactory. However, variations in type and size of tissue may re quire shorter or longer 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 may increase background staining.

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

   The amount of Solution C should be at least 7 times the tissue volume. Using a lower volume of Solution C may result in higher background staining and nonspecific precipitate.

8. Freeze tissue for sectioning with a cryostat or sliding microtome. For example, to rapidly freeze tissue:

   a. Place tissue in a plastic spoon, and slowly dip into isopentane that has been precooled with dry ice.

   The temperature should be kept below −70°C, and the dipping should take about 1 min for a mouse or rat brain (the slower the better).

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b. After the tissue is completely immersed in isopentane, place on dry ice for another minute to ensure that the tissue is well frozen.

Do not let the tissue thaw before the sections are cut.

To prevent damage to tissue caused by ice crystal formation and to preserve the best possible cell morphology, the tissue must be properly frozen before sectioning.

9. Cut 100- to 200-µm sections using a cryostat (recommended) at −20°C to −23°C.

   If the cryostat has only one temperature control, set the cryostat temperature to −23°C at least 4 hrs before cutting. If the cryostat has 2 temperature settings, set the chamber temperature 1°C colder than that of the specimen head. In most cases, −23°C is satisfactory; 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.

   a. Mount tissue on the specimen disc with distilled water, but make sure that the tissue does not thaw (may be done on dry ice).

      Tissue may also be mounted with any type of tissue freezing medium, including optimal cutting temperature (OCT) compound, but avoid cutting through the freezing medium. Do not embed the tissue in OCT. If the tissue must be embedded for cutting, use TFM™ tissue freezing medium.

      To help prevent sections from sticking to the blade, the brain surface may be coated with a thin layer of distilled water (ice) using a paintbrush. Make sure that the brain

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does not thaw at any time. The brain can be coated either before or after mounting on the specimen disc. When coating with water, the brain should be very cold so that the water freezes immediately. To coat the brain before mounting on specimen disc, first place the brain directly on dry ice. Then, dip a paintbrush in cold, deionized water, and brush the surface of the brain to apply a thin layer of water using brush strokes that are fast enough to avoid paint brush hairs sticking to the brain.

b. After tissue is mounted on the specimen disc, place tissue on dry ice for 10 min so that both the tissue and specimen disc touch the dry ice.

c. Immediately install specimen disc with tissue on the cryostat, and wait 3 to 5 min before cutting 1 or 2 sections.

Do not use the anti-roll plate.

d. Set cryostat to the thickness of sections to be cut, and cut sections.

Each type of cryostat may vary, but all types should be able to cut thick sections. If the cryostat does not have the option to set the section thickness to >60 µm, such as for 100-µm sections (or thicker), cut sections as follows: (1) Set cryostat to 50-µm section thickness. (2) Roll cryostat counterclockwise slowly, and stop when the tissue almost touches the blade (or knife). (3) Roll cryostat clockwise to cut the tissue. If the tissue is too cold (e.g., sections show cracks that are parallel to the blade), wait 1 to 2 min before the next trial. Repeat this step until high-quality sections are achieved, and then continue cutting. (4) Repeat to cut each section.

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Optional: Cut 100- to 200-µm sections with other types of microtomes, such as vibratome, sliding microtome, or rotary microtome.

If using a vibratome, the impregnated brain should be embedded in agarose or gelatin. However, for collecting sections, the cutting chamber must be filled with Solution C, otherwise sections may crack upon drying.

If using a sliding microtome, both the stage and blade need to be maintained at low temperature (<0°C).

If using a rotary microtome, tissue must be embedded in paraffin after immersion in Solution C (step 7).

10. Mount sections on gelatin-coated microscope slides using Solution C.

    a. Apply a few drops of Solution C (dropper bottle provided) onto a gelatin-coated microscope slide.

    b. Transfer sections with a glass specimen retriever (provided) onto the slide.

    c. After moving each section to an ideal location with a paintbrush, remove excess Solution C from the slide using a Pasteur pipette and a strip of filter paper if necessary.

    Any solution on the slide must be removed as much as possible; otherwise, sections may fall off the slides during staining.

    d. Allow sections to dry naturally overnight at room temperature. Do not use a fan or hot plate.

Sections should be stained as soon as possible after drying

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overnight, but unstained sections may be stored in a slide box at room temperature in the dark for up to 3 days before staining.

To prevent sections from falling off slides, properly prepared gelatin-coated microscope slides must be used (e.g., FD NeuroTechnologies, Cat. No. PO101).

VI. Staining Procedure

Do not let sections dry out between any steps during the staining or while coverslipping!

See also an instructional video for staining at www.fdneurotech.com or Current Protocols in Neuroscience, 88, e69. doi: 10.1002/cpns.69.

1. Prepare staining solution (Solution D/E), which consists of 1 part Solution D, 1 part Solution E, and 2 parts double distilled or Milli-Q water. For example, mix the following solutions in the order listed: 10 ml Solution D, 10 ml Solution E, and 20 ml double distilled water.

    - *The staining solution should be prepared immediately before use and may be used for up to 100 sections (e.g., mouse brain) per 100 ml, depending on the size of sections.*

    - *Keep the bottle and staining jar containing the staining solution covered to prevent evaporation.*

2. Rinse sections in double distilled or Milli-Q water 2 times for 4 min each rinse. Replace distilled water after each use.

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To help prevent sections from falling off slides, cold double distilled or Milli-Q water that has been stored at 4°C may be used.

3. Place sections in the staining solution (Solution D/E) for 10 min.

   Sections should be stained in a glass or plastic staining jar with sufficient solution. Do not stain sections on microscope slides.

   The staining solution should be stirred frequently during staining.

4. Rinse sections in double distilled or Milli-Q water 2 times for 4 min each rinse. Replace distilled water after each use.

   To help prevent sections from falling off slides, cold double distilled or Milli-Q water that has been stored at 4°C may be used.

5. Optional: Counterstain Golgi impregnated sections with cresyl violet.

   Note: *Counterstaining allows not only determination of the precise localization of impregnated neurons but also visualization of anatomic landmarks that would not be evident in a non-counterstained section. However, some fine dendritic branches and dendritic spines of impregnated neurons may be obscured by cresyl viole-stained cellular elements.*

   Do not let sections dry out between any steps described below.

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a. Continue to rinse sections in double distilled or Milli-Q water 2 times for 4 min each rinse. Replace distilled water after each use.

b. Place sections in 50% ethanol for 5 min.

c. Stain sections in cresyl violet solution for 20 to 30 min.

Staining time may be increased or decreased depending on the desired intensity and the concentration of cresyl violet solution used.

d. Dip sections in double distilled or Milli-Q water 3 times. Replace distilled water after each use.

e. Dehydrate sections in sequential rinses of 50%, 75%, and 95% ethanol, 30 to 50 sec each rinse, depending on the desired intensity. Do not skip any step.

The staining intensity of cellular elements and background decreases quickly in these solutions.

f. Continue to step 7.

6. Dehydrate sections in sequential rinses of 50%, 75%, and 95% ethanol, 4 min each rinse. Do not skip any step.

7. Dehydrate sections in 100% ethanol 4 times for 4 min each rinse.

Incubation may be prolonged if the section thickness is >150 µm.

8. Clear sections in xylene 3 times for 4 min each rinse. *Sections may be temporarily stored in xylene for a few hours before overslipping.*

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9. Coverslip sections with Eukitt® Quick-hardening mounting medium or Permount® mounting medium if the former is not available.

   Our recent tests indicate that the Eukitt® Quick-hardening mounting medium minimizes the development of nonspecific spherical-shaped precipitates in the background after prolonged storage and is therefore recommended for use with the FD Rapid GolgiStain™ Kit.

10. Golgi-stained sections should be stored at room temperature and protected from light.

    Golgi-stained sections should be imaged or analyzed as soon as possible. Generally, sections can be stored at room temperature in the dark for 1 year without decrease in staining intensity.

VII. Literature Cited

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