FD NeuroTechnologies, Inc.

Quality & Excellence since 1996

FD Rapid MultiStain™ Kit

An effective histological staining system with
multiple functions designed for easy use in
all types of neuroscience laboratories

User Manual

PK 501, Version 2012-01

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

FD NeuroTechnologies
Consulting & Services, Inc.

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I. Introduction

FD Rapid MultiStain™ kit, a user-friendly histological staining system, is especially designed for the morphological study of the central nervous system. The kit provides 5 most frequently used stain solutions, including hematoxylin, eosin Y, cresyl violet, neutral red and methyl green solution, all of which are ready to use straight from the bottle. FD Rapid MultiStain™ kit has been tested extensively in the tissues from both experimental animals and postmortem human brains. The solutions can be used with frozen and paraffin-embedded tissue sections as well as cultured cells (for photo samples, please visit our web site at www.fdneurotech.com).

The unique formulas of these dye solutions allow researchers who have little or no histological experience to produce the most reliable and specific staining of cellular elements with a low background. In addition, the simple procedure for each staining can be easily adopted in all types of research laboratories.

II. Kit Contents

Store at room temperature

FD hematoxylin solution	250 ml
FD eosin Y solution	250 ml
FD cresyl violet solution	250 ml
FD neutral red solution	250 ml
FD methyl green solution	250 ml
Acetic Acid Solution	250 ml
Resinous mounting medium	6 ml
Cover glass forceps	1
Disposable Pasteur Pipets	5
Rubber bulb	1
User Manual	1

III. Materials Required but Not Included

1. Distilled or deionized water
2. Histological supplies and equipment:
   - Microscope slides
   - Coverslips
   - Staining jars
   - Ethanol
   - Xylenes or xylene substitutes
   - A light microscope

IV. Safety and Handling Precautions

1. FD Rapid MultiStain™ kit is made for *in vitro* research use only and not for drug, diagnostic or other uses.
2. The kit contains reagents that may be harmful in contact with skin, by inhalation or 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.

   * Material safety data sheet is available upon request.
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.

V. Section Preparation

☑ **Note**

FD Rapid MultiStain™ kit has been proven to produce the best results in tissue sections prepared according to the following procedure. However, it may be used in tissue specimens prepared differently (please contact FD NeuroTechnologies for more information).

For perfusion-fixed tissue:

Sections of 10–30 μm thickness, depending on purposes of the experiment, may be cut on a cryostat or on a similar type of microtome, such as a sliding microtome. Sections should be either mounted directly on adhesive microscope slides or collected in 0.1 M PB (pH 7.4) and be subsequently mounted directly on adhesive microscope slides. All mounted sections should be air-dried and stored at room temperature before staining with FD Rapid MultiStain™ kit.

Note

- *To prevent tissue from ice crystal damage and to preserve the best possible cell morphology, tissue should be frozen rapidly before sectioning, e.g. by immersing the tissue in isopentane precooled to -70°C with dry ice.*
- *For the best results, Superfrost Plus microscope slides should be used for the staining with FD eosin Y solution (e.g. H&E stain).*

For unfixed frozen tissue:

Sections of 10-20 μm thickness, depending on the purpose of the experiment, may be cut on a cryostat or on a similar type of microtome, such as a sliding microtome. Sections should be directly mounted on adhesive microscope slides. After air-dried, sections should be

stored at room temperature before staining with FD Rapid MultiStain™ kit.

Note

- *To prevent tissue from ice crystal damage and to preserve the best possible cell morphology, tissue should be frozen rapidly before sectioning, e.g. by immersing the tissue in isopentane precooled to -70°C with dry ice.*
- *For the best results, Superfrost Plus microscope slides should be used for the staining with FD eosin Y solution (e.g. H&E stain).*

For paraffin-embedded tissue:

Sections of 4-10 µm thickness may be cut with a rotary microtome and placed on warm water at 40°C for 1-5 minutes. Sections should then be mounted on adhesive microscope slides and air-dried for at least overnight. Dried sections should be stored at room temperature before staining with FD Rapid MultiStain™ kit.

Note

- *The 40ºC water temperature of the flotation bath is satisfactory in most cases. However, variations in tissue type and processing may require a lower or higher temperature to obtain the best results.*
- *Superfrost Plus microscope slides should be used for the staining with FD eosin Y solution (e.g. H&E stain).*

VI. Staining Procedures:

Note

- The staining solution must be filtered before use.
- Paraffin-embedded tissue sections must be deparaffinized before staining.

Suggested protocol:

1. Place in xylene, 2 changes, 3 minutes each.
2. Place in 100% (200 proof) ethanol, 2 changes, 3 minutes each.
3. Place in 95% ethanol for 3 minutes.
4. Place in 75% ethanol for 3 minutes
5. Place in distilled water, 3 changes, 3 minutes each.
6. Continue with step 1 of the staining procedures described below for each solution except for FD hematoxylin solution (cf. note on page 10).

Warning

Xylene and ethanol are harmful or toxic to humans if ingested or inhaled. The experiment should be performed under a chemical hood with appropriate protection. Avoid contact with skin and eyes. Wear glasses and disposable gloves while doing the experiment.

FD Hematoxylin Solution (Cat. #: PS104)

FD hematoxylin solution is formulated for the staining of both neuronal and non-neuronal cellular elements. This solution can be used with frozen and paraffin-embedded tissue sections as well as cultured cells. The following procedure has been proven to produce excellent staining or counterstaining of sections from various types of tissue. However, variations in tissue type and preparation may require that the duration of steps 6 & 8 (cf. below) be shortened or lengthened to obtain the best results. The staining procedure takes approximately 1.5

hour and should be carried out at room temperature.

1. Place in xylene for 2 minutes.
2. Place in 100% (200 proof) ethanol, 2 changes, 2 minutes each.
3. Place in 95% ethanol for 2 minutes.
4. Place in 75% ethanol for 2 minutes.
5. Place in distilled water, 3 changes, 3 minutes each.
6. Stain in FD hematoxylin solution for 1-5 minutes depending on the desired intensity.
7. Rinse in 2 changes of tap water, 5 dips each.
8. Rinse in distilled water containing 2% glacial acetic acid, 5 dips (may prolong to decrease the staining intensity and the background).
9. Wash in running tap water for 20 minutes, and then rinse in distilled water.
10. Counterstain in eosin Y solution (optional).
11. Rinse briefly in 95% ethanol, 3 dips.
12. Dehydrate in 100% ethanol (200 proof), 3 changes, 2 minutes each.
13. Clear in xylene or xylene substitutes, 3 changes, 3 minutes each.
14. Coverslip in resinous mounting medium (provided).

Note
Skip steps 1-5 for paraffin sections (cf. also note on page 9).

Results:

Nuclei and basophilic cellular elements are stained blue.

FD Eosin Y Solution (Cat. #: PS103)

FD eosin Y solution is formulated for the counterstaining of tissue sections (e.g. use in combination with hematoxylin, cresyl violet, thionin or Azure II stains). This solution can be used with frozen and paraffin-embedded tissue sections. The following procedure has been proven to produce excellent results in most cases. However, variation in the type of tissue and tissue processing may require that the duration of steps 1-4 (cf. below) be shortened or lengthened to obtain the best results. The staining procedure takes approximately 30 minutes and should be carried out at room temperature.

1. Stain in FD eosin Y solution for 5-10 minutes depending on the desired intensity.
2. Rinse quickly in distilled water (may skip if too much color is lost during washing and the following alcoholic dehydration).
3. Rinse briefly in 95% ethanol, 3 dips.
4. Dehydrate in 100% (200 proof) ethanol, 4 changes, 2 minutes each (may prolong to decrease the background staining).
5. Clear in xylene or xylene substitutes, 3 changes, 3 minutes each.
6. Coverslip in resinous mounting medium (provided).

Results:

Cytoplasm is stained in various shades of pink.

FD Cresyl Violet Solution (Cat. #: PS102)

FD cresyl violet solution is formulated for the staining of both neurons and glial cells. This solution can be used with frozen and paraffin-embedded tissue sections fixed with any fixative (formalin preferred). The following procedure has been proven to produce excellent results in most cases. However, variation in the type of tissue and tissue preparation may require that the duration for steps 1, 3 and 4 (cf. below) be shortened or lengthened to obtain the best results. The staining procedure takes approximately 35 minutes and should be carried out at room temperature.

1. Stain in FD cresyl violet solution for 5-10 minutes depending on the desired intensity.
2. Rinse briefly in distilled water.
3. Differentiate in 95% ethanol containing 0.1% glacial acetic acid for 1 minute.

   Note:
   The staining intensity of both cellular elements and background decreases fast in this solution.
4. Dehydrate in 100% (200 proof) ethanol, 4 changes, 2 minutes each (may prolong to decrease the background staining).
5. Clear in xylene or xylene substitutes, 3 changes, 3 minutes each.
6. Coverslip in resinous mounting medium (provided).

Results:

Neurons and glial nuclei are stained bright blue.

FD Methyl Green Solution (Cat. #: PS105)

FD methyl green solution is formulated for the staining of both neuronal and non-neuronal cellular elements. This solution can be used with frozen and paraffin-embedded tissue sections as well as cultured cells. The following procedure has been proven to produce excellent staining or counterstaining of sections from various types of tissue. However, variations in tissue type and preparation may require that the duration of steps 1-3 (cf. below) be shortened or lengthened to obtain the best results. The staining procedure takes approximately 45 minutes and should be carried out at room temperature.

1. Stain in FD methyl green solution for 10 minutes depending on the desired intensity.
2. Rinse in 85% ethanol, 5 dips (may shorten or prolong to increase or decrease the staining intensity).

   Note:
   For counterstaining or if too much color is lost during the following alcoholic dehydration, blot slides on filter paper and let air-dry before going to the next step.

3. Dehydrate in 100% (200 proof) ethanol, 3 changes, 2 minutes each.
4. Clear in xylene or xylene substitutes, 3 changes, 3 minutes each.
5. Coverslip in resinous mounting medium (provided).

Results:

Nuclei and other basophilic cellular elements are stained bluish-green.

FD Neutral Red Solution (Cat. #: PS106)

FD neutral red solution is formulated for the staining of both neuronal and non-neuronal cellular elements. This solution can be used with frozen and paraffin-embedded tissue sections fixed with any fixative (formalin preferred). The following procedure has been proven to produce excellent results in most cases. However, variation in tissue type and preparation may require that the duration for steps 1 and 4 (cf. below) be shortened or lengthened to obtain the best results. The staining procedure takes approximately 35 minutes and should be carried out at room temperature.

1. Stain in FD neutral red solution for 5 minutes depending on the desired intensity.
2. Rinse briefly in distilled water.
3. Shake off excess water and let air-dry.
4. Dehydrate in 100% (200 proof) ethanol, 3 changes, 2 minutes each (may prolong to decrease the background staining).
5. Clear in xylene or xylene substitutes, 3 changes, 3 minutes each.
6. Coverslip in resinous mounting medium (provided).

Results:

Nuclei and other basophilic structures are stained red.

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For more references, please visit our website at www.fdneurotech.com.

Notes