FD NeuroTechnologies, Inc.

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FD Rapid TimmStain Kit™

A rapid staining kit for visualizing metals,
especially zinc-containing elements in the CNS

User Manual

PK 701, 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

Timm's sulphide silver method has so far been considered a very sensitive technique for demonstrating metal ions, in particular zinc in the central nervous system. The principle of this technique is that metals in the tissue can be transformed histochemically to metal sulphides. Subsequently, metal sulphides catalyze the reduction of silver ions by a reducing agent to metallic grains that are visible under a light or electron microscope (for review, cf. ref. 1).

Studies using this technique have provided a better understanding of not only the localization and distribution of zinc but also its possible function in the brain[1]. However, the reliability and complexity of Timm's sulphide silver staining have been major obstacles to the widespread application of this technique.

FD Rapid TimmStain Kit™ is designed based on the methods described by Haug[1] and Sloviter[2]. The reagents and procedure of the FD Rapid TimmStain Kit™ have been optimized to achieve a high degree of both specificity and sensitivity for the demonstration of metal ions, especially zinc in the brain of experimental animals. The kit can be used with both frozen and paraffin sections.

II. Kit Contents

Part 1 (Store at 4°C)

Perfusate A	500 ml
Perfusate B	500 ml
Solution D	3 ml

Part 2 (Store at -20°C)

Solution A	220 ml
Solution B	200 ml
Solution C	200 ml
Plastic forceps	1
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III. Materials Required but Not Included

1. Double distilled or Milli-Q water
2. 10% neutral buffered formalin
3. 0.1 M phosphate buffer (pH 7.4)
4. Histological supplies and equipment:
   - Superfrost Plus or gelatin-coated microscope slides
   - Coverslips
   - Staining jars
   - Xylenes or xylene substitutes
   - Resinous mounting medium (e.g. Permount®)
   - A light microscope.

Permount® is a registered trademark of Fisher Scientific.

IV. Safety and Handling Precautions

1. FD Rapid TimmStain 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 toxic or harmful in contact with skin or by inhalation and 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.

V. Tissue Preparation

The following instructions must be read before using this kit.

Note

FD Rapid TimmStain Kit™ has been proven to produce the best results in animal brain sections prepared according to the following procedure. However, it may be used in tissue specimens pre-

pared differently (please contact FD NeuroTechnologies for more information).

1. Experimental animals should be deeply anesthetized* and perfused via the ascending aorta with the perfusate (freshly made by mixing equal volumes of perfusates A and B) for 7 minutes and immediately followed by 10% buffered neutral formalin or 0.1 M phosphate buffer (PB, pH 7.4) containing 4% paraformaldehyde for 10 minutes (different species may require a different amount of the perfusate and fixative, e.g. for a mouse, 100 ml of the perfusate and 100 ml of fixative should be sufficient). Brains should be removed from the skull and postfixed in the same fixative for 24 hours at 4°C. Subsequently, brains should be transferred in 0.1 M PB containing 30% sucrose and stored at 4°C for 48 hours before freezing.

   * *Do not let animal die before the perfusion commences.*

2. Sections (20 - 40 μm in thickness) may be cut on a cryostat* or on a freezing microtome and be mounted on Superfrost Plus or gelatin-coated microscope slides. After air-drying, sections should be stored at –20°C before processing with FD Rapid TimmStain Kit™.

**** To prevent tissue from ice crystal damage and to preserve the best possible cell morphology, tissue should be frozen <u>rapidly</u> before sectioning, e.g. by immersing the tissue in isopentane precooled to -70°C with dry ice.***

ⓘ Warning

Perfusate Solutions A (contains sodium sulfide) and formaldehyde are toxic in contact with skin or by inhalation 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 THESE SOLTIONS INTO THE SINK. Collect the waste of these solutions in separate bottles and call your safety office or a licensed professional waste disposal service to dispose of these materials.

VI. Staining Procedure

- *All containers including staining jars (plastic or glass) to be used must be clean and rinsed with distilled water.*
- *Do not use metal implements during this procedure.*
- *Prepare fresh staining solution for each batch of staining.*
- *The staining solution should be protected from lights whenever possible.*

- *The following procedure should be performed at room temperaturetakes and takes approximately 2.5 hours.*

1. Wash sections in 0.1 M phosphate buffer (pH 7.4) 3 times, 3 minutes each.

   Important: *do not replace phosphate buffer with phosphate-buffered saline (PBS).*

2. Place sections in the staining solution, made freshly by mixing **Solutions A**, **B, C and D** and incubate for 50 minutes* at 30°C in the dark.

   e.g. for 100 ml:

Solution A	40 ml
Solution B	30 ml
Solution C	30 ml
Solution D	10 drops

   **50 minutes incubation time is satisfactory in most cases. However, variations in tissue processing may require that the duration of this step be lengthened or shortened to increase or decrease the staining intensity. Note that prolonging the time of incubation may also increase the background. For the best results, observe color development under a microscope frequently after 30 minutes of initial incubation. Stop the reaction by transferring sections into distill water.*

Note

- *All stock solutions should be warmed to the room temperature before mixing.*

- *Each solution should be added slowly in the order as listed while stirring.*
- *Solution D should be added just before use and well mixed with other solutions.*
- *To avoid uneven staining, sections should be incubated with gentle shaking (e.g. a shaking water bath may be used) and protected from lights whenever possible.*

3. Rinse sections in double distilled or Milli-Q water for 3 minutes in the dark.

4. Gently wash sections in running water for 30 minutes (protect from lights whenever possible).

5. Rinse sections in distilled water for 3 minutes.

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

7. Dehydrate sections in 50%, 75% and 95% ethanol, 3 minutes each (do not skip any step).

8. Dehydrate sections in absolute ethanol, 3 times, 3 minutes each (do not prolong).

9. Clear in xylene or xylene substitutes, 3 times, 3 minutes each, and coverslip in res inous mounting medium (e.g. Permount®).

VII. References

1. Haug F-M S (1973) *Heavy metals in the brain: a light microscope study of the rat with Timm's sulphide silver method. Methodological considerations and cytological and regional staining patterns.* Springer-Verlag Berlin Heidelberg New York.
2. Sloviter RS (1982) A simplified Timm stain procedure compatible with formaldehyde fixation and routine paraffin embedding of rat brain. Brain Research Bulletin 8:771-774.

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