FD NeuroSilver™ Kit II

A rapid silver staining kit for the microscopic detection of neuronal damage

User Manual

PK 301/301A, Version 2014-01

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

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I. Introduction

FD NeuroSilver™ Kit II, an improved FD NeuroSilver™ Kit I, is designed for the detection of degenerating neurons in fixed tissue sections of the central nervous system from experimental animals. The principle of this kit is based on the findings that certain components of neurons undergoing degeneration, such as lysosomes, axons and terminals, become particularly argyrophilic. Under certain conditions, these cellular elements bind to silver ions with high affinity. Upon reduction, the silver ions form metallic grains that are visible under a light or an electron microscope.

FD NeuroSilver™ Kit has been widely used in animal studies under various experimental conditions (cf. page 11 for references using this kit). This kit has proven to be very specific and sensitive for the detection of degenerating neuronal somata, axons and terminals in both the brain and the spinal cord. It is particularly useful for the detection of small numbers of degenerating neurons that may not be demonstrable with routine histopathological techniques (for photo samples, please visit our website at www.fdneurotech.com).

FD NeuroSilver™ Kit has also been proven to be sensitive and reliable for the detection of amyloid plaques in the brains of transgenic mice. In addition, this kit may be used for demonstrating neurodegeneration and/or amyloid plaques in tissue sections that have been processed for immunohistochemistry (cf. Nature Neuroscience 3: 1301-1306, 2000).

II. Kit Contents

Store at room temperature

	PK301A	PK301
Solution A	250 ml	500 ml
Solution B	250 ml	500 ml
Solution C	250 ml x 2	500 ml x 2
Solution D	250 ml	500 ml
Solution E	1 ml	2 ml
Solution F	2 ml	3 ml
Solution G (10X)	250 ml	500 ml
Glass specimen retriever	2	2
Natural hair paintbrush	1	1
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III. Materials Required but Not Included

1. Double distilled or Milli-Q water
2. 6-well tissue culture plates
3. (Optional) Corning® Netwells® inserts (Corning Cat. #3480) and carrier kit (Corning Cat. #3521)
4. Histological supplies and equipment:
   - Microscope slides and coverslips
   - Staining jars
   - Xylenes
   - Permount®
   - A light microscope.

IV. Safety and Handling Precautions

1. FD NeuroSilver™ Kit II 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 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

Note

FD NeuroSilver™ Kit II 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 prepared differently (please contact FD NeuroTechnologies for more information).

1. Experimental animals should be deeply anesthetized and perfused via the ascending aorta with 0.1 M phosphate buffer (PB, pH 7.4) for 1 minute followed by 0.1 M PB containing 4% paraformalde-

hyde for 15 minutes (different species may require a different amount of perfusates, e.g. for a rat, 100 ml of saline and 500 ml of fixative should be sufficient). Upon removal, brains should be postfixed in the same fixative as that used for the perfusion overnight at 4°C. Subsequently, brains should be immersed in 0.1 M PB containing 20% or 30% sucrose for 72 hours at 4°C before freezing.

Note

If thick sections (e.g. above 60 µm) *will be cut with a cryostat, we highly recommend the use of our tissue cryoprotection solution (Cat. #: PC102) instead of the sucrose solution.*

2. Sections of 40 – 80 µm thickness may be cut either on a cryostat* or a vibratome (similar types of microtomes, such as a sliding microtome may be used alternatively). Sections should be collected in 0.1 M PB (pH 7.4) and be subsequently transferred into 0.1 M PB containing 4% paraformaldehyde. Store sections in 0.1 M PB containing 4% paraformaldehyde for at least 7 days at 4°C before processing with FD NeuroSilver™ kit II.

**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.

ⓘ **Warning**

Paraformaldehyde is harmful and toxic to humans if ingested or inhaled. The experiment with paraformaldehyde should be performed under a chemical hood with appropriate protection. Wear glasses and disposable gloves.

VI. Staining Procedure

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 E is present.*
- *Prepare fresh working solutions and keep containers tightly closed at all times.*
- *Use 0.8 ml of solution for each 2 cm² of the section (e.g. approximately a rat brain coronal section through the dorsal hippocampus) to be stained at each step.*
- *Avoid bringing excess solution from one step to another.* ***Very important!***

- *Do not let sections dry up anytime before mounting them on slides.*
- *Use of positive control sections is recommended for each experiment.*
- *The following procedure takes approximately 1 hour and should be performed at room temperature.*

1. Rinse sections in double distilled or Milli-Q water 2 times, 5 minutes each.

2. Transfer sections into a mixture containing equal volumes of **Solutions A** and **B**, 2 times, 10 minutes each.

   Note
   - *The incubation plate must be covered during incubation to prevent splash and vaporization of the reagent.*
   - *Sections should be immersed in the solution and incubated free floating with brisk shakes.*

3. Place sections in a mixture consisting of equal volumes of **Solutions A** and **B** with **Solution E** (one drop of Solution E for each 8 ml of a total volume of Solutions A and B) for 10 minutes.

   e.g. **Solution A** 4 ml
   Solution B 4 ml
   Solution E 1 drop

   Note
   - *All 3 solutions must be well mixed and the resultant mixture should be totally clear without precipitates.*
   - *The incubation plate must be covered during incubation to prevent splash and vaporization of the reagent.*
   - *Sections should be immersed in the solution and incubated free floating with brisk shakes.*

4. Place sections in a mixture of **Solution C** and **Solution F** (one drop of Solution F for each 25 ml of Solution C), 2 times, 2 minutes each*.

   ** A total of 4 minutes of incubation time is satisfactory in most cases. However, variations in tissue processing may require that the duration of this step be lengthened (for decreasing the background stain) or shortened (for increasing the staining intensity). Note that prolonging the time of incubation will also decrease or possibly eliminate specific silver staining.*

   Note
   - *The incubation plate must be covered during incubation to prevent splash and vaporization of the reagent.*
   - *Sections should be immersed in the solution and incubated free floating with brisk shakes.*

5. Place sections in a mixture of **Solution D** and **Solution F** (one drop of Solution F for each 25 ml of Solution D) for 5 minutes.

   Note
   - *The incubation plate must be covered during incubation to prevent splash and vaporization of the reagent.*
   - *Sections should be immersed in the solution and incubated free floating with brisk shakes.*

6. Rinse sections in double distilled or Milli-Q water 2 times, 3 minutes each.

7. Place sections in 1x **Solution G** (10x concentrated solution provided, dilute with double distilled or Milli-Q water before use), 2 times, 5 minutes each.

Note

- *For the best results, keep sections in 1x Solution G at 4°C for 1-2 hours or longer if the background is high before mounting.*
- *Silver-stained sections should be protected from light whenever possible.*

8. Mount sections on pre-cleaned slides directly from 1x Solution G and let air-dry.

Note

For easy mounting, do not use adhesive microscope slides, including Superfrost Plus slides unless sections tend to fall off the slides during dehydration.

9. Clear sections in xylenes, 3 changes, 3 minutes each, and coverslip with Permount®.

Note

Do not dehydrate sections in ethanol as ethanol may cause loss of the staining.

VII. Evaluation of Results

Sections should be examined under a light microscope with either a brightfield or a darkfield condenser. Neurons undergoing degeneration are indicated by dense silver precipitates, appearing as black (brightfield) or golden (darkfield) grains, in their somata and/or processes. Photo samples are available at www.fdneurotech.com.

VIII. References using this kit:

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