NeuroTrace™ BDA-10,000 Neuronal Tracer Kit (N-7167)

Quick Facts

Storage upon receipt:
- ≤–20°C

Precautions: DAB is mutagenic. Handle with care. Once reconstituted, avoid freeze-thaw cycles for all components of this kit.

Introduction

The neuronal tracer biotin dextran amine (BDA) is transported over long distances and fills fine neuronal processes bidirectionally, including boutons in the anterograde direction and dendritic structures in the retrograde direction.[1-3] Days to weeks after BDA is injected into the desired region of the brain, the brain tissue is fixed, sectioned and incubated with avidin–horseradish peroxidase (avidin–HRP). Following a diaminobenzidine (DAB) reaction, the electron-dense DAB reaction product can be viewed by either light or electron microscopy. The NeuroTrace™ BDA-10,000 Neuronal Tracer Kit contains convenient amounts of each of the components required for neuroanatomical tracing with BDA, including:

- 10,000 MW lysine-fixable biotin dextran amine
- avidin–horseradish peroxidase
- 3,3′-diaminobenzidine

This protocol describes the injection of BDA into neural tissue, although other methods of applying the tracer to tissue are possible. The BDA labeling method can be readily combined with other anterograde or retrograde labeling methods or with immunohisto-chemical techniques.

Materials

Kit Components
- **10,000 MW lysine-fixable biotin dextran amine** (BDA, Component A), six vials, each containing 3 mg lyophilized BDA
- **avidin–horseradish peroxidase** (avidin–HRP, Component B), six vials, each containing 100 µg lyophilized avidin–HRP
- **3,3′-diaminobenzidine tetrahydrochloride, dihydrate** (DAB, Component C), one vial containing 250 mg DAB

Storage and Handling
1.1 Upon receipt, the entire kit contents should be stored frozen at –20°C or –80°C, desiccated and protected from light. When stored properly, the kit components are stable for at least six months.

1.2 BDA is supplied as a lyophilized powder containing only trace amounts of salts. This dextran conjugate should be dissolved in aqueous buffers (see *Preparation of BDA Solution*). Divide aqueous solution into aliquots and freeze at ≤–20°C. AVOID REPEATED FREEZING AND THAWING.

1.3 The peroxidase conjugate of avidin is supplied in units of 0.1 mg as a solid lyophilized from 0.1 M sodium phosphate, 0.1 M NaCl, pH 7.5. Avidin–HRP should be reconstituted in 0.1 mL distilled water to yield a 1 mg/mL stock solution (see *Preparation of Avidin–HRP Stock Solution*). Divide the aqueous solution into aliquots and freeze at ≤–20°C. When stored properly, avidin–HRP solutions are stable for at least two months. AVOID REPEATED FREEZING AND THAWING.

1.4 DAB solutions should be prepared in distilled water and then filtered through a 0.2 µm filter (see *Preparation of DAB Stock Solution*). Divide aqueous solution into aliquots and freeze at ≤–20°C. When stored properly, DAB solutions are stable for at least six months. AVOID REPEATED FREEZING AND THAWING. **Warning:** DAB is a mutagenic, tumorigenic, neoplastic compound that is potentially harmful if inhaled or ingested. DAB should be handled with appropriate precautions and disposed of in accordance with the local, state and federal regulations.

Experimental Protocol

We provide some general guidelines for neuronal tracing experiments using BDA. The accompanying Table 1 lists specific experimental conditions for seven representative BDA neuronal tracing studies from the literature. The table can be used together with this general protocol to estimate suitable conditions, including diluents, fixatives, perfusives and survival times.

Preparation of BDA Solution
2.1 Remove one 3 mg vial of BDA from the freezer.

2.2 Prepare a solution of BDA in an appropriate buffer at a suitable concentration (refer to Table 1 for specific conditions).

2.3 Divide the BDA solution into small aliquots and return all but one to the freezer.

Injection of BDA Solution into Nervous Tissue
3.1 Anesthetize the animal and access the tissue.

3.2 Inject a suitable volume of the BDA solution into the desired region of the nervous system.

3.3 Revive the animal and allow sufficient time for the dextran to travel. Suitable survival times vary depending on the experiment.

Transcardial Perfusion of the Animal

4.1 Perfuse with exsanguinate.

4.2 Perfuse with fixative.

4.3 Post-fix for 1–16 hours in fixative solution at 4°C (*optional*).

Sectioning the Nervous Tissue

5.1 Rinse the tissue to remove excess fixative.

5.2 Embed the tissue for sectioning.

5.3 Section the tissue.

5.4 Air dry the sections for at least one hour.

Preparation of Avidin–HRP Stock Solution

6.1 Remove one 100 µg vial of avidin–HRP from the freezer.

6.2 Prepare a 1 mg/mL solution of avidin–HRP by dissolving the 100 µg in 100 µL distilled water.

6.3 Divide the avidin–HRP stock solution into small aliquots and place all but one in the freezer.

Application of Avidin–HRP Solution to Tissue

7.1 Dilute an aliquot of avidin–HRP stock solution to a suitable working concentration in phosphate-buffered saline (PBS), pH 7.4, containing 0.3% Triton® X-100. A final working concentration of 0.2–1.0 µg/mL is recommended. Using a concentration as low as 0.2 µg/mL will result in lower background staining, but may provide less intense specific staining. With low concentrations of avidin–HRP, we recommend using either a nickel-enhanced DAB reaction [4] or an incubation time longer than two hours in order to enhance specific staining.

7.2 Incubate the tissue sections in the avidin–HRP working solution for 1–4 hours, or overnight if convenient.

7.3 Rinse the tissue sections three times in PBS.

Preparation of DAB Stock Solution

8.1 Remove the 250 mg vial of DAB from the freezer.

8.2 Prepare a 5% DAB solution by dissolving the 250 mg of DAB into 5 mL of distilled water. Alternatively, a smaller amount of DAB may be weighed out and dissolved in distilled water to yield a 5% solution.

8.3 Filter the DAB solution through a 0.2 µm syringe filter or filter paper.

8.4 Divide the DAB stock solution into small aliquots and place all but one aliquot in the freezer.

Application of DAB Solution to Tissue Sections

9.1 Dilute an aliquot of DAB stock solution to a final working concentration of 0.05% in distilled water. Note that when diluting a frozen aliquot of DAB solution, it may be necessary to resuspend some precipitate either by pipetting up and down or by sonicating the solution. Immediately before using the DAB working solution, add hydrogen peroxide to a final concentration of 0.003%.

9.2 Apply the DAB working solution to the tissue sections for 3–15 minutes.

9.3 Rinse the tissue sections three times with PBS.

Mounting and Storing Slides

10.1 Dehydrate the tissue sections in alcohols, clear in xylene, mount and coverslip.

10.2 Store the slides in a cool dark place.

References

1. Brain Res 607, 47 (1993); **2.** J Neurosci Methods 45, 35 (1992); **3.** J Neurosci Methods 41, 239 (1992); **4.** *Antibodies: A Laboratory Manual*, E. Harlow and D. Lane, Cold Spring Harbor Laboratory, pp 402–403 (1988); **5.** J Cell Biol 126, 901 (1994).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
N-7167	NeuroTrace™ BDA-10,000 Neuronal Tracer Kit	1 kit

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Table 1a. *Specific experimental conditions for seven representative BDA neuronal tracing studies.*

Author	***Veenman, et al*** [1]	***Veenman, et al*** [1]	***Brandt, et al*** [2]	***Wouterlood, et al*** [3] *****
Animal	White Carneaux pigeons and Wistar rats	Chick embryos at stage 29–30	rats and squirrel monkeys	Wistar rats
Injection Specifications				
Injection Region(s)	pigeons: telencephalon rats: somatosensory cortex	spinal cord	gracile nucleus and spinal cord	parietal cortex, nucleus accumbens, septum, nucleus reuniens thalami
BDA Concentration	10%	15%	10%	10%
Injection Vehicle	0.01 M PB, pH 7.25	Tyrode solution	saline	0.01 M PB, pH 7.25
Pressure Injection Method				
Injection	pigeons: micropipette tip cemented to a 1 µL Hamilton microsyringe rats: NA	pneumatic pressure injection system	pneumatic pressure injection system	NA
Micropipette Tip Diameter	pigeons: 20–50 µm rats: NA	10–20 µm	50 µm	NA
Injection Amount	pigeons: 0.05–0.1 µL (0.01 µL steps/min) rats: NA	multiple small injections	0.2–0.5 µL	NA
Iontophoretic Injection Method				
Current	positive-pulsed DC current 2–5 µA, 7s on and 7s off for 30–60 min	NA	positive-pulsed DC current 5–7 µA, 0.1 Hz for 20 min	positive-pulsed DC current 5 µA, 7s on and 7s off for 20 min
Micropipette Tip Diameter	20–50 µm	NA	30 µm	20 µm
Tissue Processing				
Survival Time	6–11 days	NA	rats: 7, 14, and 23 days; monkeys: 3, 5, and 7 weeks	6 days
Exsanguinate	0.2 mL 1.2% heparin in avian saline, then 30–50 mL 6% dextran in 0.1 M PB, pH 7.4	NA	saline	100 mL saline, pH 6.9, 38ºC
Fixation	4% Pf + 0.01 M sodium periodate + 0.1 M PB, pH 7.4	(immersion) 2% GA, 1.25% GA + 1% Pf or 0.5% GA + 3% Pf for 3 hr, then 0.1 M PB, pH 7.4, 4°C	4% Pf + 0.1 M AB, pH 4.5, then chilled 4% Pf + 0.05% GA + 0.05 M BB, pH 9.5, then 10% sucrose + PB, pH 7.6, 4ºC	4% FA + 0.1% GA + 0.2% picric acid + 0.125 M PB, pH 7.3
Cryoprotection	20% sucrose + 10% glycerol + 0.02% sodium azide + 0.1 M PB, pH 7.4	10% sucrose + 0.1 M PB, pH 7.4, then store in 20% sucrose + 0.1 M PB, pH 7.4, 4ºC	20% sucrose + PB, 4°C	NA
Sectioning Method	sliding microtome, 40 µm frozen sections	cryostat, 30 µm sections	freezing microtome, 50 µm sections	Vibratome, 40 µm sections in cold 0.125 M PB

***** This is a protocol for electron microscopy and the details are too extensive to be included in its entirety. **NA** = Not applicable.
AB = acetate buffer **BB** = borate **DMSO** = dimethylsulfoxide **FA** = formaldehyde **GA** = glutaraldehyde
Pf = paraformaldehyde **PB** = phosphate buffer **PBS** = phosphate buffered saline **TBS** = Tris-buffered saline **Triton** = Triton X-100

References
1. J Neurosci Methods 41, 239 (1992); **2.** J Neurosci Methods 45, 35 (1992); **3.** J Neurosci Methods 48, 75 (1993); **4.** J Comp Neurol 340, 161 (1994); **5.** J Neurosci Methods 51, 9 (1994); **6.** Brain Behav Evol, 46, 108 (1995).

Table 1b. *Specific experimental conditions for seven representative BDA neuronal tracing studies.*

Author	***Code & Carr*** [4]	***Dolleman-van der Weel, et al*** [5]	***Matz*** [6]
Animal	White leghorn chicks	Wistar rats	Chinook salmon
Injection Specifications			
Injection Region(s)	inner ear	entorhinal cortex and nucleus reuniens thalami	olfactory bulb and telencephalon
BDA Concentration	5–10%	10%	3% or 10%
Injection Vehicle	PBS + 0.02% Triton	0.01 M PB, pH 7.4	PBS
Pressure Injection Method			
Injection	Hamilton syringe	NA	micropipette tip attached to a 1 µL Hamilton microsyringe
Micropipette Tip Diameter	not specified	NA	20–50 µm
Injection Amount	0.3–0.6 µL (0.5 µL/2 min)	NA	0.05–0.2 µL
Iontophoretic Injection Method			
Current	NA	positive-pulsed DC current 5–6.5 µA, 7s on and 7s off for 20 min	NA
Micropipette Tip Diameter	NA	15–20 µm	NA
Tissue Processing			
Survival Time	2–10 days	7–10 days	1–7 days
Exsanguinate	0.9% saline	200 mL 0.9% saline	20 mL PBS
Fixation	4% Pf	4% Pf + 0.05% GA + 0.1 M PB, pH 7.4	4% Pf + 0.5% GA, then post fix 1–4 hr in 4% Pf + 0.5% GA
Cryoprotection	0.1 M PB, then 30% sucrose, PB	2% DMSO, 20% glycerin, PB, pH 7.4	PBS, imbed in agar, then sink in 30% sucrose
Sectioning Method	25–50 µm frozen sections, collected in PBS	freezing microtome, 40 µm in 0.05 M TBS, pH 7.6	cryostat, 15–30 µm sections

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AB = acetate buffer **BB** = borate **DMSO** = dimethylsulfoxide **FA** = formaldehyde
GA = glutaraldehyde **Pf** = paraformaldehyde **PB** = phosphate buffer **PBS** = phosphate buffered saline
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