For Research Use Only.
Not for use in diagnostic procedures.

Acetylcholinesterase Rapid Staining Kit

Code No.	Quantity
8450	30 tests

BACKGROUND :

Acetylcholinesterase (AChE) is a tetrameric serine hydrolase that rapidly catalyzes the hydrolysis of acetylcholine to acetate and choline. The breakdown of acetylcholine is critical for the termination of impulse transmissions at cholinergic synapses within the nervous system. Progressive loss of cholinergic neurons in Alzheimer's disease (AD) patients results in severe memory loss and impairment of cognitive function. AChE inhibitors are a strategic approach to symptomatic treatment for AD, since AChE inhibitors increase the levels of acetylcholine in the synapse, thereby enhancing cholinergic activity in the affected regions of the brain. AChE also plays an important role in agriculture since modifications in AChE can confer resistance to pesticides. AChE is a key component in many snake venoms, and AChE staining is routinely used for the initial diagnosis of Hirschsprung's disease, a congenital disorder caused by the absence of ganglion cells in the distal colon.

STORAGE: All kit components must be stored at 4°C. This product is stable for 1 year from the date of manufacture.

Expiration: Please see the label on the kit box.

PRODUCT COMPONENTS:

Solution A-1	3 mL x 1 vial
Solution A-2	3 mL x 1 vial
Solution B	6 mL x 1 vial
DAB solution	600 µL x 1 vial
H_2O_2 solution	600 µL x 1 vial
DAB dilution buffer	24 mL x 1 bottle
Disposal droppers	30 droppers

PROTOCOL:

1) Prepare 4 µm air-dried cryostat sections.
2) Rinse the slides with tap water for 10 seconds.

[optional]
Endogenous peroxidase quenching may be required. If necessary, immerse sections in methanol containing 0.3% H_2O_2 for10 minutes.

3) Prepare "Solution A mixture" by mixing Solution A-1 and A-2 by 5 drops each.
4) Add 10 drops of Solution B to "Solution A mixture".
5) Immediately drop the mixed solution prepared in step 4) onto the sections using a disposable dropper pipette.
6) Incubate the slides at 37°C for 3-10 minutes.
7) Incubate the slides at 37°C for 3-10 minutes.
8) Rinse the slides with tap water for 10 seconds.
9) Add one drop of H_2O_2 solution to 800 µL of DAB dilution buffer. Mix well and add one drop of DAB solution.
10) Immediately drop the mixed solution prepared in step 8) onto the sections using another disposable dropper pipette.
11) Development for 5 minutes at room temperature.

[optional]
Counter staining with hematoxylin may be performed after washing with tap water.

12) Wash the slides with tap water, dehydrate and mount.

REFERENCES:

1) Foudi, A., *et al., J. Exp. Med.* **211**, 909-927 (2014)
2) Hai, B., *et al., Clin. Cancer Res.* **20**, 140-150 (2014)
3) Kobayashi, H., *et al., Pediatr. Surg. Int.* **23**, 505-508 08 (2007)
4) Kobayashi, H., *et al*., *Pediatr. Surg. Int.* **18**, 349-353 (2002)
5) Kobayashi, H., *et al*., *Arch. Pathol. Lab. Med.* **118,** 1127-9 (1994)

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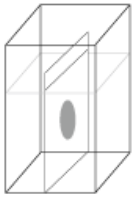
1

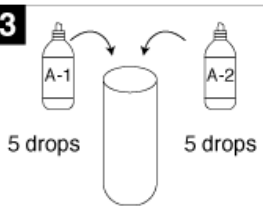
Prepare 4 μm air-dried cryostat sections.

2



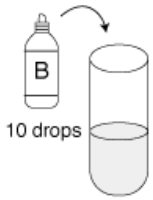
Rinse the slides with tap water for 10 seconds.

3



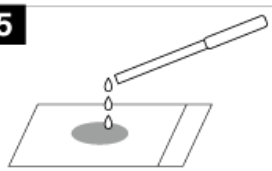
Prepare "Solution A mixture" by mixing Solution A-1 and A-2 by 5 drops each.

4



Add 10 drops of Solution B to "Solution A mixture" .

5

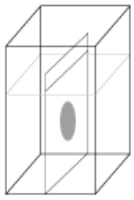


Immediately drop the mixed solution onto the sections using a disposable dropper pipette.

6

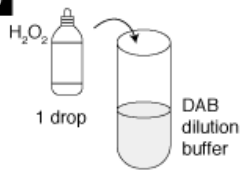
Incubate the slides at 37°C for 3-10 minutes.

7



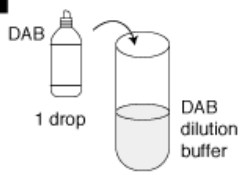
Rinse the slides with tap water for 10 seconds.

8



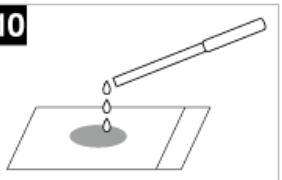
Add one drop of H_2O_2 solution to 800 μL of DAB dilution buffer.

9



Mix well and add one drop of DAB solution.

10



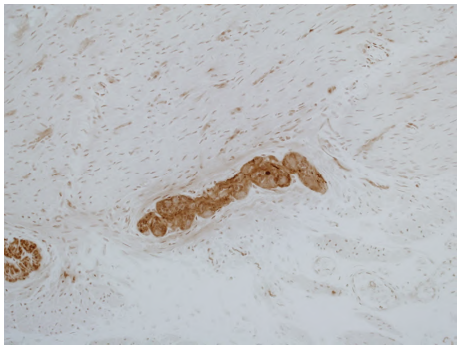
Immediately drop the mixed solution prepared onto the sections using another disposable dropper pipette.

11

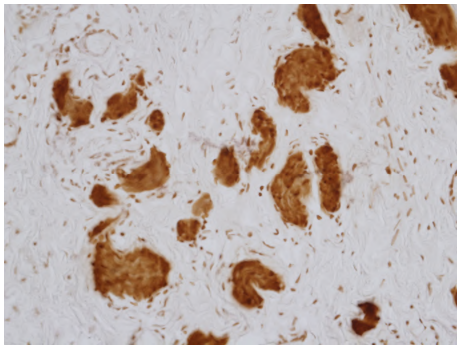
Development for 5 minutes at room temperature.

12

Wash the slides with tap water, dehydrate and mount.



Myenteric ganglia in normal intestine (x 200)



Submucosal hypertrophic nerve trunks in Hirschsprung's disease (x 400)

The photos are kindly provided by Dr. Hiroyuki Kobayashi at Juntendo University.