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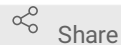
# Post mortem human substantial nigra TH staining

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1 Works for me



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## ABSTRACT

This protocol is standardized for postmortem (frozen) SN tissue from pathologically diagnosed PD patients and control individuals for Immunofluorescence staining.

## DOI

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## PROTOCOL CITATION

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## KEYWORDS

ASAPCRN

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## CREATED

Feb 18, 2022

## LAST MODIFIED

Jul 12, 2022

MATERIALS TEXT

Anti-TH, Sigma T1299, 1:400

Secondary antibody - goat anti-mouse Alexafluor 488, Lifetech, Cat# A11001) 1:400

ProLong Gold antifade (Lifetech, P36935)

## 1 Deparaffinization

6m

Wash slides in 2 changes of **300 mL** ml of xylene – **00:06:00** each at room temperature.

## 2 Rehydration

23m

Wash slides in **300 mL** of 100% alcohol – **00:09:00** at **Room temperature**.

Wash slides in **300 mL** of 95% alcohol – **00:06:00** at **Room temperature**.

Wash slides in **300 mL** of 80% alcohol – **00:03:00** at **Room temperature**.

Rinse slides in gentle running distilled water – **00:05:00** at **Room temperature**.

## 3 Antigen retrieval

30m

Microwave (high power) slides in **200 mL** **0.01 Molarity (M)** sodium citrate buffer, pH 6.0 at **99-100 °C** for **00:05:00**, three rounds.

Add **40 mL** distill water after 1<sup>st</sup> round

Add **150 mL** sodium citrate buffer after the 2<sup>nd</sup> round.

Let stand at room temperature in the buffer - **00:20:00**

Rinse in 1X TBS with Tween (TBST) – for **00:05:00** at **Room temperature**.

## 4 Immunostaining

1h 10m

**Do not allow tissues to dry at any time during the staining procedure.**

Apply a universal protein block (5% goat serum in TBST) – for **00:30:00** at **Room temperature**.

Drain protein block from slides, and apply diluted primary antibody overnight at **4 °C**.

Rinse slides in 1X TBST 3 times - **00:05:00** each at **Room temperature**.

Incubate secondary antibody (Conc - 1:400) - **00:30:00** at **37 °C**.

Rinse slides 1X TBST 3 times – **00:05:00** each at **Room temperature**

Mount the slides with ProLong Gold antifade