Alpha-synuclein immunochemistry on STC-1 cells using DAB

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We use this protocol and it's working

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ABSTRACT

This protocol describes how to visualise alpha-synuclein in STC-1 cells by DAB immunohistochemistry. It also works for other antibodies (e.g. 5-HT, CCK, GLP).

MATERIALS

Rabbit anti-alpha-synuclein	ab212184	Abcam
Rabbit anti-beta-actin	ab241153	Abcam
Goat anti-rabbit IgG biotin	111-065-144-JIR	Stratech
Streptavidin	POD11089153001	Roche

1 Grow STC-1 cells on 12 mm glass coverslips treated with poly-D-lysine or Geltrex™.

2 Fix cells with 4 % paraformaldehyde 00:15:00 15m

Safety information

Paraformaldehyde is toxic by inhalation
158127 (sigmaaldrich.com)

3 Wash cells with PBS **2** x 00:05:00 5m

4 Aspirate and discard PBS and freeze cells < -20 °C

5 Thaw frozen 24-well plates containing fixed cells grown on glass coverslips

6 Wash cells with PBS 00:05:00 5m

7 Quench with 0.3% H_2O_2 00:05:00 5m

8 Wash cells with PBS **2** x 00:05:00 5m

9 Block with 10 % goat serum in PBS containing 0.005 % Triton™ X-100 and 0.05 % thimerosal 01:00:00 1h

10 Incubate with rabbit anti-alpha-synuclein (1:500) (Abcam, ab212184) in 10 % goat serum containing 0.005 % Triton™ X-100 and 0.05 % thimerosal (block buffer) 20:00:00 20h

11 Wash with PBS **4** x 00:05:00 5m

12 Incubate with biotinylated goat anti-rabbit IgG (1:500) (Stratech, 111-065-144-JIR) in 2 % goat serum containing 0.005 % Triton™ X-100 and 0.05 % thimerosal (antibody buffer) 02:00:00 2h

13 Wash with PBS **4** x 00:05:00 5m

14 Incubate with 1:250 streptavidin-HRP (Roche, 11089153001) for 1 hour in antibody buffer 01:00:00 1h

15 Wash with PBS **6** x 00:05:00 5m

16 Visualise staining by incubation with PBS containing 0.05 % (w/v) 3,3'-Diaminobenzidine/0.015 % (v/v) H_2O_2/0.05 % (w/v) nickel ammonium sulphate for ≤ 00:03:00 3m

17 Rinse for > 00:05:00 in running tap water 5m

18 Remove coverslips from wells and mount on microscope slides with Hydromount™ (National Diagnostics)