**D2 Dopamine Receptor IHC Protocol**

Adapted from:

K.A. Neve, C.P. Ford, D.C. Buck, D.K. Grandy, R.L. Neve, T.J. Phillips, Normalizing dopamine D2 receptor-mediated responses in D2 null mutant mice by virus-mediated receptor restoration: Comparing D2L and D2S, Neuroscience, Volume 248, 17 September 2013, Pages 479-487, ISSN 0306-4522, http://dx.doi.org/10.1016/j.neuroscience.2013.06.035.

(http://www.sciencedirect.com/science/article/pii/S0306452213005411)

Day 1:

1. In 12-well plate, wash desired slices 1 x 5 min in 1X PBS (2 mL)
2. Hydrogen peroxide incubation: 15 min at room temperature

PBS

0.3% H2O2

1. Wash 3 x 5 min in PBS
2. Non-specific block: 4 hours at room temperature

PBT (0.25% Triton-X in 1X PBS)

5% goat serum

1. Primary antibody incubation: overnight at room temperature

PBT

0.1% BSA

1:5000 primary (Millipore/Chemicon # AB5084P, Rabbit IgG polyclonal)

Day 2:

1. Wash slices 3 x 5 min in PBS
2. Secondary antibody incubation: 2 hours at room temperature

1:200 biotinylated goat anti rabbit IgG (Vectastain)

PBT

0.1% BSA

1. Wash slices 3 x 5 min in PBS
2. ABC incubation: 1 hour at room temperature

VECTASTAIN® ABC immunoperoxidase system PK6100 (Vector Labs, Burlingame CA, USA)

1. Wash slices 3 x 5 min in PBS
2. DAB incubation: 3 minutes at room temperature (Themo Scientific, Rockford, IL, USA)
3. Wash slices in water