**Immunohistochemistry Protocol**

**Materials**

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| **Reagent** | **Supplier** | **Cat. #** |
| Phosphate Buffered Saline, pH 7.4 | Millipore-Sigma | P3813-10PAK |
| Triton X-100 | Millipore-Sigma | T9284-100ML |
| Normal Donkey Serum | Jackson ImmunoResearch | 017-000-121 |
| ProLong Diamond Antifade Mountant | Fisher Scientific | P36970 |

**Preparation**

1. Make up 1 L of fresh Phosphate Buffered Saline (PBS) from 10x stock solution
2. Make up PBS-T: 0.2–0.5% Triton X-100 in PBS (stir 100–250 mg Triton in 50 mL of PBS)

**Primary reactions**

1. Place each series in a glass vial and rinse mouse brain sections with PBS 3 times before starting primary reactions.
2. Add 1 mL of PBS-T with 2% Normal Donkey Serum (20 µL) to each series and swirl briefly.
3. Optional blocking step: leave slices in PBS-T and 2% Normal Donkey Serum at room temperature for 45–60 min.
4. Add primary antibody to each series.
5. Shake gently for 48 h at 4 *◦*C (sections should barely revolve around the vial).

**Secondary reactions**

1. Rinse sections with PBS 3 times before starting secondary reactions.
2. Add 1 mL of PBS-T with 2% Normal Donkey Serum (20 µL) to each series and swirl briefly.
3. Add secondary antibody to each series.
4. Shake gently for 90 min at room temperature, protected from light (sections should barely revolve around the vial).
5. Rinse sections with PBS 3 times before mounting.
6. Mount sections serially on slides with Prolong Diamond Anti-fade mounting media; protect slides from light and keep at 4 *◦*C after 24 h drying at room temperature.