

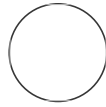


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Immunohistochemistry Protocol

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ABSTRACT

This protocol describes immunohistochemistry.

ATTACHMENTS

[729-1840.docx](#)

MATERIALS

Materials

A	B	C
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

OPEN ACCESS

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Protocol status: Working
We use this protocol and it's working

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PROTOCOL integer ID:
82682

Keywords:
immunohistochemistry



Phosphate buffered saline powder, pH 7.4, for preparing 1 L solutions Merck MilliporeSigma (Sigma-Aldrich) Catalog #P3813



Triton X-100 Merck MilliporeSigma (Sigma-Aldrich) Catalog #T9284






Normal Donkey Serum Jackson ImmunoResearch Laboratories, Inc. Catalog #017-000-121



ProLong Diamond Antifade Mountant Thermo Fisher Scientific Catalog #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

- 3 Place each series in a glass vial and rinse mouse brain sections with PBS 3 times before starting primary reactions.



- 4 Add  1 mL of PBS-T with 2% Normal Donkey Serum ( 20 µL) to each series and swirl briefly.



- 5 **Optional blocking step:** leave slices in PBS-T and 2% Normal Donkey Serum at  Room temperature for 45–60 min.



- 6 Add primary antibody to each series.



- 7 Shake gently for  48:00:00 at  4 °C (sections should barely revolve around the vial).



2d

Secondary reactions

1d 1h 30m

8 Rinse sections with PBS 3 times before starting secondary reactions.






9 Add  1 mL of PBS-T with 2% Normal Donkey Serum ( 20 μ L) to each series and swirl briefly.







10 Add secondary antibody to each series.



11 Shake gently for  01:30:00 at  Room temperature , protected from light (sections should barely revolve around the vial).  1h 30m

12 Rinse sections with PBS 3 times before mounting.



13 Mount sections serially on slides with Prolong Diamond Anti-fade mounting media; protect slides from light and keep at  4 °C after  24:00:00 drying at  Room temperature .  1d