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## Immunohistochemistry Protocol

DOI

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**Protocol status:** Working

We use this protocol and it's working

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## Abstract

This protocol describes immunohistochemistry.

## Attachments




[729-1840.docx](#)

19KB

## 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

 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**


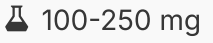

 Normal Donkey Serum **Jackson ImmunoResearch Laboratories, Inc. Catalog #017-000-121**

 ProLong Diamond Antifade Mountant **Thermo Fisher Scientific Catalog #P36970**


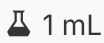
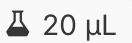




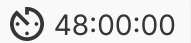
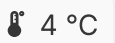

## Troubleshooting



## Preparation






- 1 Make up  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  Triton in  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  of PBS-T with 2% Normal Donkey Serum (  ) to each series and swirl briefly. 
- 5 **Optional blocking step:** leave slices in PBS-T and 2% Normal Donkey Serum at  for 45–60 min. 
- 6 Add primary antibody to each series. 
- 7 Shake gently for  at  (sections should barely revolve around the vial). 

## Secondary reactions

1d 1h 30m

- 8 Rinse sections with PBS 3 times before starting secondary reactions. 
- 9 Add  of PBS-T with 2% Normal Donkey Serum (  ) 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