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Marmoset NHP Free Floating Anti-GFP Antibody Stain After HCR FISH

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ABSTRACT

A protocol for staining NHP tissue for anti-GFP antibody after staining for markers of interest with HCR FISH.

PROTOCOL CITATION

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protocols.io
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MATERIALS

NAME	CATALOG #	VENDOR
Goat serum	G9023	Sigma Aldrich
GFP Polyclonal Antibody	A-11122	Invitrogen - Thermo Fisher
Goat anti-Rabbit IgG (H L) Highly Cross-Adsorbed Secondary Antibody Alexa Fluor 488	A-11034	Invitrogen - Thermo Fisher

Primary Antibody

3d

- 1 Permeabilized marmoset brain sections (\approx **100 μ m** , fixed previously in **4 % volume** PFA) in **1.2 % volume** Triton X-100 in PBS for **00:30:00** .
- 2 Block sections with **10 % volume** NS (natural serum depending on Secondary Antibody) and PBT [**0.1 % volume** Triton X-100, 1X PBS] for **01:00:00** .
- 3 Incubate primary antibody (1:500 whole mount - **0.002 % volume**) with **3 % volume** NS and PBT-Azide [**0.2 % volume** NaN₃, 1X PBT] at **4 °C** for **72:00:00** .

Secondary Antibody

2d

- 4 Wash sections in 1X PBS for ⌚ 00:10:00 6X.
- 5 Incubate with secondary antibody (1:500 whole mount - [M].002 % volume) in PBT at ⚡ 4 °C for ⌚ 24:00:00 .
- 6 Wash sections in wash buffer for ⌚ 00:10:00 6X.
- 7 Stain for DAPI (1:10000 in PBS - [M].0001 % volume).
- 8 Wash sections in 1X PBS for ⌚ 00:05:00 2X.