

Immunofluorescence Protocol

A general immunofluorescence protocol for the Bevan lab

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Materials

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

Procedure

Preparation

1. Make up 1 L of fresh Phosphate Buffered Saline (PBS) from powder sachet
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 sections with PBS 3 times before starting primary reactions
4. Add 1 mL of PBS-T with 2% Normal Donkey Serum (20 μ L) 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 (see table 2)
7. Shake gently for 48 h at 4 °C (sections should barely revolve around the vial)

Secondary reactions

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 (see table 3)
11. Shake gently for 90 min at room temperature, protected from light (sections should barely revolve around the vial)
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 h drying at room temperature

Antibodies

Table 2: Primary antibodies

Name	Host	Supplier	Cat#	Dilution (/1 mL)
Anti-NeuN (A60)	Mouse	Millipore-Sigma	MAB377	1:200 (5 μ L)
Anti-NeuN (EPR12763)	Rabbit	Abcam	ab177487	1:1000 (1 μ L)
Anti-Tyrosine Hydroxylase (LNC1)	Mouse	Millipore-Sigma	MAB318	1:500 (2 μ L)
Anti-Huntington Protein (mEM48)	Mouse	Millipore-Sigma	MAB5374	1:100 (10 μ L) ¹
Anti-GFAP (polyclonal)	Chicken	Abcam	ab4674	1:1000 (1 μ L)
Anti-Iba1 (polyclonal)	Rabbit	Wako	019-19741	1:1000 (1 μ L)
Anti-c-Fos (9F6)	Rabbit	CST ²	2250S	1:500 (2 μ L)
Anti-Parvalbumin (polyclonal)	Guinea	SynapticSystems	195 004	1:1000 (1 μ L)
Anti-vGluT1 (polyclonal)	Guinea	SynapticSystems	135304	1:1000 (1 μ L)
Anti-vGluT2 (polyclonal)	Rabbit	SynapticSystems	135403	1:1000 (1 μ L)
Anti-vGAT (117G4)	Mouse	SynapticSystems	131011	1:250 (4 μ L)
anti-Gephyrin (mAb7a)	Mouse	SynapticSystems	147021	1:300 (3.33 μ L)
Anti-Bassom (polyclonal)	Guinea	SynapticSystems	141004	1:1000 (1 μ L)
Anti-GABA _A -R γ 2 (polyclonal)	Rabbit	SynapticSystems	224003	1:1000 (1 μ L)

Table 3: Secondary antibodies

Fluorophore	Host	Target	Supplier	Cat#	Dilution (/1 mL)
Alexa 488	Donkey	Anti-Mouse	Jackson ImmunoResearch	715-545-150	1:250 (4 μ L)
Alexa 568	Donkey	Anti-Mouse	Fisher Scientific	A10037	1:333 (3 μ L)
Alexa 594	Donkey	Anti-Mouse	Jackson ImmunoResearch	715-585-150	1:250 (4 μ L)
Alexa 647	Donkey	Anti-Mouse	Jackson ImmunoResearch	715-605-150	1:250 (4 μ L)
Alexa 488	Donkey	Anti-Rabbit	Jackson ImmunoResearch	711-545-152	1:250 (4 μ L)
Alexa 568	Donkey	Anti-Rabbit	Fisher Scientific	A10042	1:333 (3 μ L)
Alexa 594	Donkey	Anti-Rabbit	Jackson ImmunoResearch	711-585-152	1:250 (4 μ L)
Alexa 647	Donkey	Anti-Rabbit	Jackson ImmunoResearch	711-605-152	1:250 (4 μ L)
Alexa 488	Donkey	Anti-Chicken	Jackson ImmunoResearch	703-545-155	1:250 (4 μ L)
Alexa 594	Donkey	Anti-Chicken	Jackson ImmunoResearch	703-585-155	1:250 (4 μ L)
Alexa 647	Donkey	Anti-Chicken	Jackson ImmunoResearch	703-605-155	1:250 (4 μ L)

¹We've also tested the anti-htt at 1:150 and it looks to work OK

²Cell Signaling Technology