Cascade Blue - tdTomato - VGluT2 Triple Staining (acute)

0. 神経細胞にtdTomatoを発現した脳から急性スライスを作製する。

1. パッチ電極に0.1%Cascade Blue hydrazide, 3Li塩を含め、20分程度ホールセル記録後を保つ ([Stuart & Sakmann, 1994](#_ENREF_3))。

2. 細胞から静かに電極を外す。

3. 24穴wellプレートに移し、PB wash

4. 4%PFA and 0.2%Picric acid 0.1MPB, ON at 4°C

以下室温、遮光

5. 10%Sucrose in PB, 30 min ([Kawaguchi & Kubota, 1996](#_ENREF_1))

6. 20%Sucrose in PB, 1 hr

7. Freeze and thaw with dry ice, three times

8. PBS wash

9. 10%NGS in PBS-X, 30 min

10. 1st antibody in PBS-XG, ON (振盪)

11. PBS-X wash

12. 2nd antibody in PBS-XG, 2 hr (振盪)

13. PBS-X wash

14. PBS wash

15. 2% gelatin, 15 min

16. スライドガラスにマウント

17. 50% (v/v) glycerol/2.5% (w/v) triethylene diamine in PBSまたはVECTASHIELD Hard setで封入

18. 観察

Slice: 　　　　　　 　 　　Date:

<切片プレート>

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 |
| A |  |  |  |  |  |  |
| B |  |  |  |  |  |  |
| C |  |  |  |  |  |  |
| D |  |  |  |  |  |  |

**溶液**

**Pipette solution**

8% cascade blue stock 6.25 µl (final 0.1%)

Cs-based patch solution 500 µl

**PBS-X** (total 550 ml程度)

20% Triton X-100 2.75 ml final 0.1%

in PBS 547.25 ml

**PBS-XG** (150 ml程度)

normal goat serum 1.5 ml (final 1%)

sodium azide 30 mg (final 0.02%)

in PBS-X 148.5 ml

**10%NGS in PBS-X** (5 ml程度)

normal goat serum 0.45 ml (final 10%)

in PBS-XG 4.55ml

**1st antibodies** ([Miyazaki *et al.*, 2003](#_ENREF_2)) (200倍希釈、原液200 µg/ml)

VGluT2 (guinea pig anti-VGluT2, frontier science, VGluT2-GP-Af670)

in PBS-XG

**2nd antibody** (1000倍希釈) (原液 2 mg/ml) (1–1000 µg/ml final推奨)

Alexa Fluor 633-labeled goat anti-guinea pig IgG (A-21105)

in PBS-XG

**Mounting medium**

250 mg triethylene diamine

5 ml glycerol

5 ml PBS

**Reagents**

VGluT2-GP-Af670: すべて20 µg, \36,750

A-21105: 0.5 ml, \41,000

VECTASHIELD Hard set: H-1400, 10 ml, \18,000

**2 % Gelatin液**　　　　（50°Cに温めて溶かし、室温に冷ましてから使用）

Gelatin 0.4 g

in PBS 20 ml

**文献**

Kawaguchi, Y. & Kubota, Y. (1996) Physiological and morphological identification of somatostatin- or vasoactive intestinal polypeptide-containing cells among GABAergic cell subtypes in rat frontal cortex. *J Neurosci*, **16**, 2701-2715.

Miyazaki, T., Fukaya, M., Shimizu, H. & Watanabe, M. (2003) Subtype switching of vesicular glutamate transporters at parallel fibre-Purkinje cell synapses in developing mouse cerebellum. *Eur J Neurosci*, **17**, 2563-2572.

Stuart, G.J. & Sakmann, B. (1994) Active propagation of somatic action potentials into neocortical pyramidal cell dendrites. *Nature*, **367**, 69-72.