Calcium imaging of ReproNeuro Ver1.1

Provided from
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Schedule

day	-1	0	3	7	14	…	21
Coarting of plate	●						
Preparation of medium		●					
Thawing and plating		●					
Exchange medium			●	●	●		●
Assay					●	●	●

Required reagents

- ReproNeuro [ReproCELL, RCESDN001,RCESDN002,RCDN002N,RCDN003P]
- ReproNeuro Maturation Medium [RCESDN301]
- Hanks' Balanced Salt Solution (HBSS) [Life Technologies Corporation, 14025-076]
- Dimethyl Sulfoxide (DMSO) [Nacalai, 13407-45]
- Fluo-8 (Ex/Em : 490 nm/514 nm) [ABD Bioquest, 21081]
  (Make Fluo-8 (5 mM) solution in DMSO. Aliquote and stock in deep freezer.)
- 35-mm-diam dish with grid [Matsunami, D111505]

Instruments for observation

・Confocal laser scanning microscopes [Olympus, FLUOVIEW FV1000 IX81]

・Objective lens [Olympus, UPLSAPO 40 x oil]

Procedures

1. Plate ReproNeuro

1.1. Coat a 35-mm-diam dish according to the manual.

1.2. Plate ReproNeuro on the coated dish (cell density is $7.5x10^4$ cells/cm^2). Cell suspension is placed on only glass region. After half day, add maturation medium in the dish.

1.3. Change half volume of medium at day 3, 7, 14, 21.

1.4. Assay is recommended to perform between day 14 and 21.

2. Loading Fluo-8

2.1. To make Fluo-8 (5 μM) solution, add 2μL of Fluo-8 (5 mM) solution into 200 μL of Maturation medium.

2.2. Remove the cultured medium from to the dish and then add 200 μL of the Fluo-8 (5 μM) solution.

2.3. Incubate the dish at 37℃ for 40 min.

2.4. Remove the cultured medium from to the dish and then add 2 mL of HBSS.

2.5. Remove the cultured medium from to the dish and then add 2 mL of HBSS.

2.6. Remove the cultured medium from to the dish and then add 2 mL of HBSS.

2.7. Incubate the dish at 37℃ for 15 min and then observed the cells.

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