Calcium imaging of ReproNeuro cells

ReproNeuro RCDN002N, RCDN003P, RCDN001N
(Also discontinued items RCESDN001 & RCESDN002)

Version 2.0

Schedule

day	-1	0	3	7	14	…	21
Plate coating	●						
Medium preparation		●					
Thawing cells and plating		●					
Fresh Medium Exchange			●	●	●		●
Assay					●	●	●

Required Reagents and Supplies

Name	Catalog Number	Note
ReproNeuro Culture Medium	RCDN101	Store at 4°C
Hank's Balanced Salt Solution	ThermoFisher Corp, (GIBCO) Cat.No. 14025-076	Store at 4°C
Dimethyl Sulfoxide (DMSO)	Nacalai Japan Cat.No. 13407-45	
Fluo-8 [Ex/Em: 490nm/514nm]	ABD Bioquest Cat.No. 21081	Make 5mM in DMSO; store -70°C
35 mm non-coated dish with grid	Matsunami Glass Cat.No. D111505	see diagram on next page
0.01% Poly-L-Lysine (PLL) Solution	Sigma Cat.No. P4832	Store at 4°C
ReproNeuro Coat	Reprocell, RCDN201	Store at 4°C

Instruments for Observation

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

・Objective lens [Olympus, UPLSAPO 40 x oil]

Procedures

1. Plate ReproNeuro cells

1.1. Prepare 5 mL of PLL coating solution as described in **section 1** of the *Protocol of ReproNeuro*™. Coat the 35 mm dish (glass portion) with 425 uL volume of 0.0033% of PLL at 37°C for 2 hours.

1.2. Just prior to the next step, prepare the ReproNeuro Coat solution by mixing 1 mL of PBS and 30 uL of ReproNeuro Coat in a sterile 15 mL conical tube.

1.3. Retrieve the plate from the 37°C incubator. Remove the PLL solution and apply 425 uL of fresh PBS to the plate for a rinse. Remove and discard the rinse. Repeat the rinse one more time.

1.4. Add 425 uL volume of the previously prepared ReproNeuro Coat solution to the glass region of the plate. Incubate at 37°C overnight.

1.5. The next day, thaw and prepare ReproNeuro cells according to the manual, up to **step 4.12**. The cell concentration at this step is 2.0×10^5 cells/ mL. (See *Protocol of ReproNeuro*™).

1.6. Remove the ReproNeuro Coat solution from the plate and immediately plate 425 uL prepared ReproNeuro cells on the coated dish (cell density is 7.5×10^4 cells/cm^2). Place the cell suspension only on the glass region (1.13 cm^2 area). Incubate covered dish at 37°C overnight for about 8-12 hours to allow attachment.

1.7. Remove 200 uL of the culture medium and exchange for fresh Culture medium (200 uL).

1.8. Repeat this exchange of half volume (200 uL) of Culture medium at day 3, 7, 14, 21.

1.9. It is recommended to perform the calcium imaging assay between day 14 and 21.

2. Calcium Imaging Assay

2.1. The Fluo-8 working concentration is 5 μM in Culture medium. To prepare the reagent, add 2 ml of Culture medium into a sterile 15 ml conical tube. Add 2 μL of Fluo-8 (5 mM) reagent into the tube containing the Culture medium. The concentration is now 5 μM and ready to apply to the cells.

2.2. Remove the cultured medium from to the dish and then add 425 μL of the freshly prepared Fluo-8 (5 μM) solution.

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

2.4. Remove the Fluo-8 (5 μM) solution from to the dish and then add 2 mL of HBSS to rinse the entire dish.

2.5. Remove the HBSS (salt solution rinse) from the dish and then again add 2 mL of HBSS.

2.6. Remove the HBSS (salt solution rinse) from the dish and then again add 2 mL of HBSS.

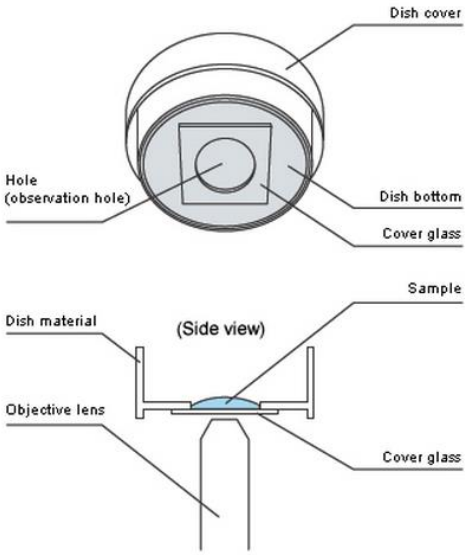
Note: You have now completed 3-washes

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

Matsunami Glass Industries, Ltd.

Website: http://www.matsunami-glass.co.jp/life/index.html

Figure 1. Schematic of the Matsunami Glass company 35mm glass bottom dish which is suitable for Calcium Imaging of ReproNeuro cells.



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