

Resource Summary Report

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CULTURING i3NEURONS (Basic Protocol 6)

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Protocol Information

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Group: Neurodegeneration Method Development Community

Summary: Following Day 3 replating, i3Neurons should be cultured for at least 1 week before lysis, fixation, or other experimental endpoint, and they remain viable for at least 1 month with proper maintenance. This protocol covers medium conditions, coating of tissue culture dishes with synthetic polymers, recommended plating densities, and maintenance procedures for long-term culture and for specific experimental applications. Cortical Neuron Culture Medium (CM) is sufficient to promote the maturation and longterm maintenance of i3Neurons in culture. i3Neurons express general markers of cortical neurons, as well as specific pre- and postsynaptic markers of glutamatergic excitatory cortical neurons. Since these cells are post-mitotic after 3 days of differentiation (see Basic Protocol 5) and prefer neuron-conditioned medium to fresh medium, maintenance conditions for these cultures are minimal. Generally, half-medium changes every 7 days with fresh, pre-warmed CM are sufficient for culturing beyond d10 (7 days after replating). Neuron attachment and growth also require a strongly adhesive substrate. Coating plates with synthetic polymers such as poly-L-ornithine (PLO) is necessary for i3Neuron attachment, viability, and successful outgrowth.

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