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TRANSFECTION OF i³NEURONS (Support Protocol 3)

In 1 collection

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ABSTRACT

Transient protein expression can easily be studied in i³Neurons using lipid-based transfection. This protocol is identical to that in iPSCs (see [Basic Protocol 2](#)). i³Neurons are modestly transfectable, with 5 % to 10 % of cells showing fluorescent protein expression after 24 hr. We have found that refreshing neuronal medium 1 to 2 hr after transfection both allows successful DNA entry into cells and largely prevents cytotoxicity resulting from the transfection reagent. Unlike iPSCs, i³Neurons show increased protein expression/accumulation over time, with greater fluorescence 48 to 72 hr after transfection than at 24 hr. Transient transfections also show more durable expression in i³Neurons than iPSCs, likely because episomes are not diluted by cell division. i³Neurons can be transfected in suspension (i.e., replating after day 3 of differentiation) or as an adherent culture, although better results are observed in adherent cultures. They are also amenable to serial transfections (i.e., re-transfecting with the same construct 24 hr apart) if higher-percentage transfections are desired.

ATTACHMENTS

[fernandopulle2018.pdf](#)

Protocol status: Working
We use this protocol and it's working

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Keywords: i3LMN,
i3Neurons, iPSC, iPSC-derived
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mediated differentiation

GUIDELINES

This protocol is identical to that in iPSCs (see [Basic Protocol 2](#)). i³Neurons are modestly transfectable, with 5 % to 10 % of cells showing fluorescent protein expression after 24 hr. We have found that refreshing neuronal medium 1 to 2 hr after transfection both allows successful DNA entry into cells and largely prevents cytotoxicity resulting from the transfection reagent. Unlike iPSCs, i³Neurons show increased protein expression/accumulation over time, with greater fluorescence 48 to 72 hr after transfection than at 24 hr. Transient transfections also show more durable expression in i³Neurons than iPSCs, likely because episomes are not diluted by cell division. i³Neurons can be transfected in suspension (i.e., re-plating after day 3 of differentiation) or as an adherent culture, although better results are observed in adherent cultures. They are also amenable to serial transfections (i.e., re-transfecting with the same construct 24 hr apart) if higher-percentage transfections are desired.

SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.