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# TRANSFECTION OF i$^3$NEURONS (Support Protocol 3)

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

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**Keywords:** i3LMN, i3Neurons, iPSC, iPSC-derived neurons, transcription factor-mediated differentiation

## Abstract

Transient protein expression can easily be studied in i$^3$Neurons using lipid-based transfection. This protocol is identical to that in iPSCs (see **Basic Protocol 2**). i$^3$Neurons 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, i$^3$Neurons 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 i$^3$Neurons than iPSCs, likely because episomes are not diluted by cell division. i$^3$Neurons 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.

## Attachments

PDF

fernandopulle2018.pd...

1.7MB

## Guidelines

This protocol is identical to that in iPSCs (see **Basic Protocol 2**). i$^3$Neurons 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, i$^3$Neurons 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 i$^3$Neurons than iPSCs, likely because episomes are not diluted by cell division. i$^3$Neurons 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.