Tau Seed Detection via FRET Flow Cytometry

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BRIEF DESCRIPTION:

This assay was designed to detect small amounts of tau seeds in biological samples, such as brain homogenates from human and rodent tauopathy models. HEK-293T cells stably expressing a human tau sequence were engineered to serve as biosensors of intracellular tau aggregation in a fluorescence resonance energy transfer (FRET)-based assay. Specifically, the biosensor cells express a tau repeat domain (RD) sequence containing the P301S mutation tagged to either cyan fluorescent protein (CFP) or yellow fluorescent protein (YFP). At baseline, this tau protein is maintained in a soluble, monomeric form and is FRET negative. Upon introduction of tau seeds to the biosensor cells, the tau protein aggregates, and a FRET response can be sensitively and quantitatively measured using flow cytometry. This assay is compatible with multiple seed sources, including recombinant and synthetic tau seeds, as well as brain homogenate.

****** Tau FRET biosensor cells will be made available via ATCC in the coming months.

PROTOCOL:

1) Replate FRET biosensor cells:

1a) Detach cells from a 10cm plate with 0.05% trypsin, and spin down at 1000 x g. Resuspend cell pellet in complete media (DMEM + 10% FBS + 1% Pen/Strep + 1% GlutaMax).

1b) Count cells, and plate 35,000 cells per well of a 96 well plate. Each well should contain 130 µL media.

- Four cell lines are used: HEK293T cells; CFP single-positive cells,YFP single-positive cells, and CFP/YFP dual-positive cells. For a full 96 well plate, dedicate 4 wells each to HEK 293T, CFP, and YFP cells. The remainder of the plate is dedicated to CFP/YFP cells.

1c) Incubate overnight.

2) Treat FRET biosensor cells:

**When cells are 60% confluent, transduce tau seeds with liposome preparations.

2a) Make transduction complexes. Ideal transduction complexes are made by combining [8.75 µL Opti-MEM + 1.25 µL Lipofectamine 2000] and [Opti-MEM + tau seeds] such that a total volume to be added per well is 20 µL.

2b) Incubate transduction complexes for 20 minutes at room temperature before adding to cells.

2c) Incubate overnight.

3) FRET Flow Cytometry:

**Use a FRET-compatible flow cytometer that will capture 1) CFP excitation → CFP emission 2) YFP excitation → YFP emission and 3) CFP excitation → YFP emission. We use a MACSQuant VYB (Miltenyi).

3a) 24 hours after treatment, detach cells with trypsin and spin down at 1000 x g. Resuspend and postfix cell pellet in 2% paraformaldehyde for 10 minutes.

3b) Spin down at 1000 x g and resuspend cell pellet in flow cytometry buffer (HBSS plus 1% FBS and 1 mM EDTA).

3c) On the flow cytometer, use HEK 293T cells to set up live/dead cell gates and singlet/doublet gates. Ensure that CFP, YFP, and FRET emission signals are negative in this cell population.

3d) Use CFP single-positive cells to eliminate CFP bleed-through into the YFP and FRET channels. Using compensation methods, ensure that the median fluorescent intensity (MFI) of the YFP and FRET channels is equivalent between HEK 293T cells and CFP single-positive cells.

** The compensation step can be omitted from this protocol.

3f) Following setup, run remaining wells, capturing 20,000 events from the singlet gate.

4) Data Analysis:

4a) Use vehicle-treated (lipofectamine-only) cells to set up live/dead cell gates and singlet/doublet gates.

4b) Use YFP single-positive cells to construct a "false FRET" gate (FRET vs YFP on bivariate plot). This gate should be drawn such that YFP bleed-through into the FRET channel is removed.

4c) Use vehicle-treated cells to set up a FRET gate. A triangular gate running close to the slope of the cell population and extending upward and leftward will define FRET positivity. Percent positivity should be approximately 1% in vehicle-treated cells. (See Figure 1 below).

4d) Within the FRET gate, determine the percentage of FRET-positive cells and the mean fluorescence intensity (MFI) of all FRET-positive cells.

4e) Report the Integrated FRET Density, which is the product of percent positive cells and MFI of FRET-positive cells.

Figure 1