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Cell-based Tau seeding assay

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Abstract

This protocol details the procedure of cell-based Tau seeding assay.

Attachments

d5m9biezx.pdf

511KB

Guidelines

Banning, C. et al. A flow cytometry-based FRET assay to identify and analyse proteinprotein interactions in living cells. PLoS One 5, e9344, doi:10.1371/journal.pone.0009344 (2010).

Materials

TauRD-YT cell line: HEK293T cell line stably co-expressing the repeat domain of Tau (TauRD; residues 244-372 with FTD mutations P301L/V337M) fused to YFP or mTurquoise2.
FLTau-YT cell line: HEK293T cell line stably co-expressing FLTau (0N4R with FTD mutations P301L/V337M) fused to YFP or mTurquoise2.

Troubleshooting

1 Plate 100,000 cells per well of HEK293T FRET reporter cell line (TauRD-YT, FLTau-YT or commercial cell line Tau RD P301S FRET Biosensor (ATCC, CRL- 3275)) into a 12-well plate.

Note

NOTE:
TauRD-YT cell line: HEK293T cell line stably co-expressing the repeat domain of Tau (TauRD; residues 244-372 with FTD mutations P301L/V337M) fused to YFP or mTurquoise2.
FLTau-YT cell line: HEK293T cell line stably co-expressing FLTau (0N4R with FTD mutations P301L/V337M) fused to YFP or mTurquoise2.

2 On the next day, seed aggregation in cells with Tau aggregates.

Note

Aggregates can be added directly to the media or transfection reagent can be used to circumvent cellular uptake pathways.

2.1 **Seeding with transfection reagent:**

- Prepare lipofectamine mix for n+1 samples: 50 µL Opti-MEM (Gibco) + 1.6 µL lipofectamine 3000 reagent (Thermo Fisher Scientific) per sample.
- Distribute 51.6 µL of the lipofectamine mix to individual tubes.
- Add corresponding amount of Tau aggregates.

Note

NOTE: For Tau seeding-competence quantification, Tau aggregate concentration should be determined experimentally by aggregate titration. Increasing amounts of aggregates should be added to the cells. Choose a concentration within the linear range of seeding. Tentative concentrations are:

- When using TauRD-YT cells as reporter cell line and TauRD aggregates produced in vitro (Tau residues 244-371, C291A/P301L/C322A/V337M, see protocol Tau aggregation reaction and thioflavin-T (ThT) fluorescence measurements), seeding with 14 ng TauRD aggregates (10 µL of a 1:100 dilution in PBS of the aggregation reaction at 10 µM) results in around 10-20% FRET positive cells.
- When using TauRD-YT cells as reporter cell line and FLTau (aggregates produced in vitro (FLTau 0N4R, see protocol Tau aggregation reaction and thioflavin-T (ThT) fluorescence measurements), seeding with 183 ng FLTau aggregates (40 µL of a 1:100 dilution in PBS of the aggregation reaction at 10 µM) results in 10-20% FRET positive cells.

NOTE: When lysates from cells containing aggregates are used as seeding material, lyse cell pellets with 0.05% Triton X-100/PBS, Complete EDTA-free protease inhibitor cocktail (Merck) and benzonase for 20 min on ice. Quantify total protein by Bio-Rad Protein Assay (Bio-Rad) or Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). The amount of Tau protein can be quantified by SDS-PAGE and immunoblotting using purified Tau as standard or by ELISA. Tentative concentrations are:

- When using TauRD-YT cells as reporter cell line and lysate from TauRD-YT cells containing aggregates, seeding with 1 µg total protein (around 8 ng TauRD-YFP and TauRD-mTurquoise2) results in 2-5% FRET positive cells. The residual concentration of Triton-X-100 in the seeds added to the cells is around 0.01%.
- When using TauRD-YT cells as reporter cell line and lysate from FLTau-YT cells containing aggregates, seeding with 10 ng total protein (around 200 pg FLTau-YFP and FLTau-mTurquoise2) results in 7% FRET positive cells. The residual concentration of Triton-X-100 in the seeds added to the cells is around 0.0001%.

2.2 Seeding without transfection reagent:

- Mix Tau aggregates with 500 µL fresh media.
- Replace cell media with this mix.

Note

NOTE: Tentative concentrations: When using TauRD-YT cells as reporter cell line and TauRD aggregates produced in vitro (Tau residues 244-371, C291A/P301L/C322A/V337M, see protocol Tau aggregation reaction and thioflavin-T (ThT) fluorescence measurements), seeding with 400 ng TauRD aggregates results in around 2-3% FRET positive cells after 48 h incubation.

3 Collect and quantify FRET positive cells after 16:00:00 - 20:00:00 (when using lipofectamine) or 48:00:00 (without lipofectamine or when using the FLTau-YT reporter cells): 3d 12h

3.1 Wash cells with PBS.

3.2 Add 100 µL TrypL Express Enzyme (Gibco).

3.3 Collect the cells adding 400 µL of media and transfer them to an Eppendorf tube.

3.4 Centrifuge at 1000 x g for 00:05:00 . 5m

3.5 Discard the supernatant and wash the cell pellet with 200 µL PBS.

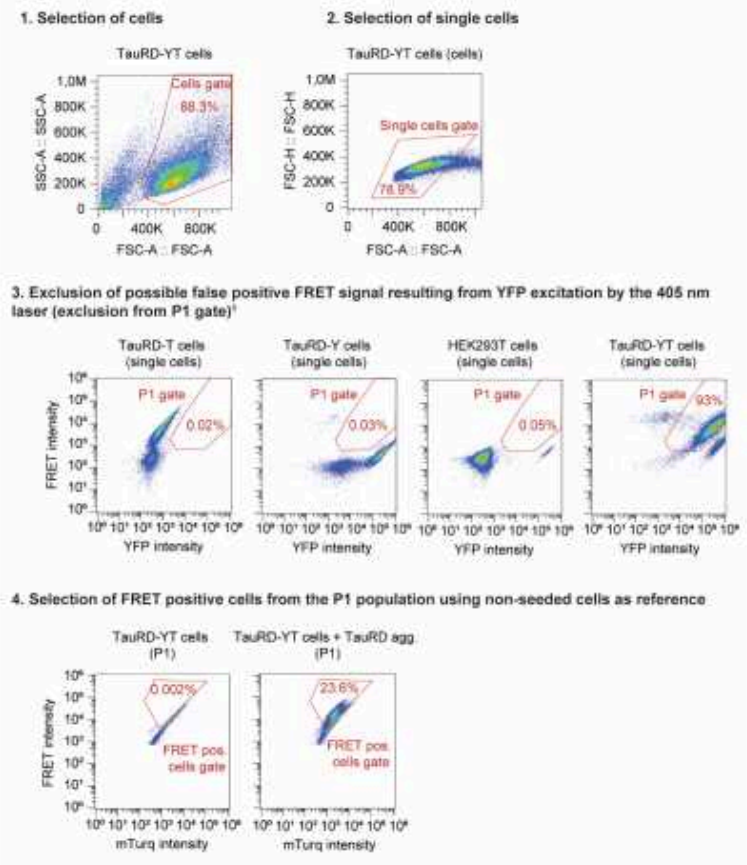
3.6 Centrifuge at 1000 x g for 00:05:00 . 5m

3.7 Discard supernatants and resuspend the cell pellet in 200 µL PBS.

Note

NOTE: Cells can be fixed after collection and stored at 4°C until quantification. After the PBS wash, resuspend the pellet with 400 µL 4% paraformaldehyde (PFA) and incubate at room temperature for 10 min. Centrifuge at 1000x g for 5 min, resuspend the pellet in PBS and store at 4°C.

3.8 Quantify FRET positive cells by flow cytometry. A minimum of 50,000 single cells should be analyzed. The FRET positive gate is set by plotting the FRET fluorescence signal versus the mTurquoise2 fluorescence signal using as reference nonseeded cells (See Figure below).



Flow cytometry gating strategy for quantification of FRET positive cells. To measure the mTurquoise2 and FRET fluorescence signals, cells were excited with 405 nm laser light and fluorescence was determined using 440/50 and 530/30 filters, respectively. To measure the YFP fluorescence signal, cells were excited at 488 nm and emission was recorded using a 530/30 filter. For each sample, 50,000 single cells were analyzed. First, cells were selected (1), followed by single cell selection (2). After gating single cells, an additional gate (P1) was introduced to exclude YFP-only cells that show a false-positive signal in the FRET channel due to excitation at 405 nm using as reference TauRD-T cells, TauRD-Y cells and HEK293T cells[1] (3). The FRET positive gate was set by plotting the FRET fluorescence signal versus the mTurquoise2 fluorescence signal using as reference non-seeded cells (4). **Supplementary Fig. 10 from Yuste-Checa P., et al Nat commun xxx 2021).**

Note

NOTE: When using TauRD-YT or FLTau-YT cells as reporter cell line, cells expressing just Tau-YFP and cells expressing just Tau-mTurquoise2 should also be analyzed as controls. After gating single cells, an additional gate is introduced to exclude YFP-only cells that show a false-positive signal in the FRET channel due to excitation at 405 nm1. (See figure below for gating strategy).
NOTE: An Attune NxT flow cytometer (Thermo Fisher Scientific) can be used with the following settings:
mTurquoise2: Excitation 405 nm - Emission 440/50.
FRET: Excitation 405 nm - Emission 550/30.
YFP: Excitation 488 nm - Emission 550/30.