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Cytotoxicity BioAssay of human iPSC-derived Dopamine Neurons (DANs)

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We use this protocol and it's working

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Abstract

This protocol details a sensitive, fast, high-throughput and non-destructive cytolysis assay that measures adenylate kinase (AK) release from damaged cells. It detects AK in cell culture supernatants, providing real-time, kinetic analysis of cell death without requiring cell lysis of iPSCs-derived DANs plated in a 96-well format plate.

Materials

Reagents:

- Phosphate-buffered saline (PBS), pH 7.4 (Life Technologies, CAT# 10010056)
- Triton X-100 (Sigma- Aldrich, SKU# X100)
- AK assay buffer

Equipment:

- ToxiLight™ Non-Destructive Cytotoxicity BioAssay Kit (Lonza Biologics PLC, CAT# LT07-217)
- Lumitrac™ White 384 well Microplate (Greiner Bio-One Ltd, CAT# 781075)
- PHERAstar® microplate reader (BMG Labtech)

SOLUTIONS

Prepararing 20% Triton X-100 solution:

1. Add 2 mL of Triton X-100 solution in 10 mL PBS.

Prepararing Detection Reagent:

1. Reconstitute the lyophilised AK detection reagent by adding 10 mL of AK assay buffer.

2. Allow it to sit for 15 mins before use.

Before start

Cells should be plated at a density of 50 000 cells/ well in a 96 full area plate which has been precoated with Geltrex.

Additionally, care should be taken when adding and removing liquid as these adherent cells are prone to peeling.

Avoid exposure to light by covering the plate with aluminium foil.

Record the record within 5 minutes of adding the reagent to the supernatant.

1 Collect 5 µl of cell supernatant and transfer to a 384-well Greiner LUMITRAC™ plate.

2 Add 25 µl of detection reagent to each well and keep it for 5 minutes.

3 Record luminescence was using a PHERAstar® microplate reader.

4 For positive control, add an equal volume of 20% Triton X-100 solution to the media of one well per cell line for 5 minutes before supernatant collection.