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Apoptosis Detection with CellEvent Caspase-3/7

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We use this protocol and it's working

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Abstract

Protocol describing methods used in Palumbos et al., 2025 to assay cell death in primary cortical neurons following exosomal inhibition. Approach combines CellEvent Caspase 3/7 reporter with Cellpose machine learning detection. Expanded methods applying these two approaches individually are available (see Invitrogen directions and Stinger and Pachitariu 2025).

Culture Neurons to DIV 7

1 On DIV0, plate 200k primary cortical neurons into 4 wells/condition of 12-well plate with glass-like bottom polymer (P12-1.5P, Cellvis).

Note

This can be done with any cell type. This protocol use culturing and dissection conditions as described in https://dx.doi.org/10.17504/protocols.io.81wgby723vpk/v1

2 Culture neurons with standard protocol until DIV7.

3 Prepare imaging media and warm in 37°C incubator with CO2.

Note

Imaging media- Hibernate E + B27 + Glucose
Need 4mL per well to be imaged.

Add Drugs to Plates

4 Prepare drug aliquots for concentration gradient to be added to 1mL maintenance media

Note

If no drug conditions are being used, skip steps 4-6

4.1 GW4869 (0µM, 1µM, 2.5µM, 5µM final concentration in 1mL maintenance media).

5 Add drug aliquots to corresponding well(s). Be sure to properly label

6 Return to 37°C incubator for 2 hours.

Prepare for Imaging

7 Prepare imaging media + CellEvent™ Caspase-3/7. For 4mL, add 1 drop of CellEvent™ Caspase-3/7 (R37111)

8 Warm media at least 30min in 37C waterbath

9 Wash plates 1X with prewarmed imaging media (no dye added)

10 Add 1mL of imaging media +CellEvent™ Caspase-3/7 per well.

11 Add 1 µL of Hoechst per plate.

12 Immediately image, capturing DIC, 405, 488. Capture 10 fields of view per condition. Fields of view should be selected throughout the plate, without biasing based on caspase signal.

> Note
>
> This is experiment was conducted on a Leica DMI6000B inverted epifluorescence microscope equipped with a filter wheel for high-speed imaging and a climate-controlled chamber.

12.1 Do not select field of view based on 488 signal, but rather using nuclear signal.

12.2 Capture 1 z-plane focusing on 405 signal. If you are also capturing DIC signal, this will likely require a separate z-plane.

Analysis Using Cell Pose

13 Create separate folders per channel (e.g., 405).

14 Open Cellpose3 (Stinger and Pachitariu 2025).

Note

Cellpose3 (Stinger and Pachitariu 2025) has thorough independent documentation that would be beneficial for other cell types and conditions.

15 Load folder containing images.

16 Check auto-adjust saturation.

17 Check- MASK ON, outlines on, single stroke.

18 Set nuclear size.

18.1 Segmentation- set diameter to 30 pixels.

Note

Other cell types or imaging conditions will have to adjust diameter size. Record all settings before moving forward with other images

19 Select model.

19.1 Other models- custom models- nuclei.

20 Run Cyto3.

Note

Before continuing with entire dataset, parameters should be adjusted based on signal, nuclear size, and confluency

21 Record ROIs counted.

22 Repeat steps 11-19 with CellEvent Caspase signal (488 channel folder)

23 All cell event ROIs should correspond with nuclear signal. To determine percent of nuclei that express CellEvent, take CellEvent ROI number divided by Hoescht ROI number.

Protocol references

Stringer, C., Pachitariu, M. Cellpose3: one-click image restoration for improved cellular segmentation. *Nat Methods* **22**, 592–599 (2025). https://doi.org/10.1038/s41592-025-02595-5

CellEvent™ Caspase-3/7 Detection Reagents