Oct 31, 2022

Immunocytochemical analysis

In 1 collection

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dx.doi.org/10.17504/protocols.io.bp2l69bmrlqe/v1

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ABSTRACT

Protocol for the immunocytochemical analysis of cell culture cells.

DOI

dx.doi.org/10.17504/protocols.io.bp2l69bmrlqe/v1

PROTOCOL CITATION

Felix Kraus 2022. Immunocytochemical analysis. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bp2l69bmrlqe/v1

COLLECTIONS

Kraus et al., 2022 FBXO7 /Park15

CREATED

Oct 11, 2022

LAST MODIFIED

Oct 31, 2022

PROTOCOL INTEGER ID

71172

Seeding of HeLa

1 Wash HeLa cells with 1x PBS

2 Add Trypsin to cells for 5 min and incubate at 37°C to dissociate cells from plastic well

3 Resuspend cells in 1 mL DMEM media

4 Count cells

5 Seed appropriate number of cells into 6-/12-/24-well glass bottom dish

6 Top up glass bottom dish with either 3-/1.5/1 mL DMEM and place cells back into incubator

7 The next day exchange DMEM with DMEM + 2µg/ml doxycycline for 18h to induce Parkin expression.

8 Induce mitophagy using Antimycin A / Oligomycin A for the desired time.

Staining

9 Aspirate DMEM and fix cells in 1 ml pre-warmed 4% PFA for 30 min.

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10 Aspirate PFA solution and wash wells 3x with PBST (1x PBS, 0.02% Tween 20)

11 Permeabilize the cells by adding 0.2% Triton X-100 in PBS.

12 Remove the detergent solution by aspiration. Wash wells 3x with PBST (1x PBS, 0.02% Tween 20). Drain well.

13 Block cells for 10 min with 3% BSA – 1x PBS.

14 Remove BSA solution by aspiration. Wash wells 3x with PBST (1x PBS, 0.02% Tween 20). Drain well.

15 Incubate with primary antibodies in 3% BSA - 1x PBS for 3h at RT or overnight at 4°C with gentle shaking.

16 Wash wells 3x with PBST (1x PBS, 0.02% Tween 20). Drain well.

17 Incubate with secondary antibodies in 3% BSA - 1x PBS for 45 min – 1h.

18 Wash wells 3x with PBST (1x PBS, 0.02% Tween 20). Drain well.

19 If required, add Hoechst33342 or DAPI 1:2000 to wells for 5 min with gentle shaking.

20 Wash wells 3x with PBST (1x PBS, 0.02% Tween 20). Drain well.

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21 Exchange PBST with 1x PBS and keep cells at 4°C until imaging. Image within the next few days.

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