JUL 31, 2021

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.bsr6nd9e

Protocol Citation: Odetta Antico, Miratul M. Muqit 2021. PROCEDURE TO ISOLATE AND CULTURE NEURONS FROM EMBRYONIC MOUSE CORTEX . **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bsr6nd9e>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
 We use this protocol and it's working

Created: Feb 25, 2021

Last Modified: Dec 26, 2022

PROTOCOL integer ID:
 47646

Keywords: Neurons, PINK1, Parkin, Mitochondrial stress, ubiquitin, ASAPCRN

PROCEDURE TO ISOLATE AND CULTURE NEURONS FROM EMBRYONIC MOUSE CORTEX

In 1 collection

Odetta Antico¹, Miratul M. Muqit¹

¹Medical Research Council Protein Phosphorylation and Ubiquitylation Unit, School of Life Science, University of Dundee, Dow Streets, Dundee, DD1 5EH, UK

ASAP2020

alessi

m.muqit

ABSTRACT

Mutations in PINK1 cause early-onset Parkinson's disease. PINK1 becomes stabilised and active upon mitochondrial depolarisation. This leads to phosphorylation of ubiquitin and Parkin via Serine 65 residues and a feed forward mechanism whereby PINK1 phosphorylates newly formed polyubiquitin chains, generating phospho-ubiquitin, which further promotes Parkin recruitment and activation. Once activated, Parkin ubiquitylates proteins at the outer face of the outer mitochondrial membrane (OMM) and then initiates a downstream pathway that eventually leads to mitophagy, a mitochondria-specific type of autophagy. Notably, much of previous investigation into PINK1/Parkin activity has been performed in non-neuronal human cancer cells where Parkin and/or PINK1 is over-expressed. Here we report a protocol for generation of mouse embryonic cortical neuronal cultures that produce high cell yields and can be used for studying endogenous PINK1 and Parkin signalling by biochemical methods and proteomics.

ATTACHMENTS

[Mouse PINK1 pathway protocol \(166 - 337\).pdf](#)

MATERIALS

For culture

1. E16.5 mouse embryos (8-10 embryos, either sex; we routinely use C57BL/6j mice or PINK1 wild-type and knockout mice)

CRITICAL All experiments must be conducted in accordance with the relevant institutional and governmental guidelines and regulations.

2. Dissection medium:

HBSS, calcium, magnesium, no phenol red **Gibco - Thermo Fisher Catalog #14025050**

3. Digestion medium:

Trypsin-EDTA **Gibco - Thermo Fisher Catalog #25300054**

DNase I **Merck MilliporeSigma (Sigma-Aldrich) Catalog #11284932001**
in HBSS

A	B
Trypsin-EDTA	0.025%
DNase I	0.125 mg/mL

4. Dissociation medium:

Neurobasal™ Medium **Gibco - Thermo Fisher Catalog #21103049**

Fetal Bovine Serum qualified heat inactivated Brazil **Gibco - Thermo Fisher Catalog #10500064**

B-27™ Supplement (50X) serum free **Gibco - Thermo Fisher Catalog #17504044**

GlutaMAX™ Supplement **Gibco - Thermo Fisher Catalog #35050061**

A	B
Neurobasal medium	
Foetal Bovine Serum (FBS) heat-inactivated	10%

A	B
B27 supplement, serum free	1X
GlutaMAX supplement	1%

5. Culturing medium:

⊗ Neurobasal™ Medium **Gibco - Thermo Fisher Catalog #21103049**

⊗ B-27™ Supplement (50X) serum free **Gibco - Thermo Fisher Catalog #17504044**

⊗ GlutaMAX™ Supplement **Gibco - Thermo Fisher Catalog #35050061**

A	B
Neurobasal medium	
B27 supplement, serum free	1X
GlutaMAX supplement	1%

6. ⊗ Poly-L-lysine hydrobromide **Merck Catalog #P2636**

7.

⊗ Water sterile-filtered BioReagent suitable for cell culture **Merck Catalog #W3500**

8.

⊗ Trypan Blue solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T8154**

For biochemistry

1. Mitochondrial depolarisation:

🧪 10 undetermined

⊗ Antimycin A from Streptomyces sp. **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A8674**

🧪 1 undetermined

⊗ Oligomycin A **Merck MilliporeSigma (Sigma-Aldrich) Catalog #75351** in DMSO

Dimethyl sulfoxide **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D265**

2. Lysis Buffer:

cOmplete™ EDTA-free Protease Inhibitor Cocktail **Roche Catalog #11873580001**

2-Chloroacetamide **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C0267**

A	B
Tris-HCl (pH 7.5)	50 mM
Sucrose	250 mM
EDTA	1 mM
EGTA	1 mM
Sodium orthovanadate	1 mM
Sodium β -glycerophosphate	10 mM
Sodium fluoride	50 mM
Sodium pyrophosphate	5 mM
TritonX	1% (w/v)
Protease inhibitor cocktail supplemented with 200 mM chloroacetamide	

3.

DPBS no calcium no magnesium **Gibco - Thermo Fischer Catalog #14190094**

4.

Coomassie Protein Assay Reagent **Thermo Scientific Catalog #1856209**

5.

NuPAGE™ LDS Sample Buffer (4X) **Invitrogen - Thermo Fisher Catalog #NP0008**

6.

2-Mercaptoethanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M6250**

7.

PageRuler™ Prestained Protein Ladder 10 to 180 kDa **Thermo Fisher Scientific Catalog #26616**

8.

Immobilon-P PVDF Membrane **Merck Catalog #IPVH00010**

9.

Amersham™ Protran® Western blotting membranes
nitrocellulose **Merck Catalog #GE10600041**

10.

NuPAGE™ 4 to 12% Bis-Tris 1.0 mm Mini Protein Gel 10-well **Invitrogen - Thermo Fisher Catalog #NP0321BOX**

NuPAGE™ 4 to 12% Bis-Tris 1.0 mm Midi Protein Gel 20-well **Invitrogen - Thermo Fisher Catalog #WG1402BOX**

11.

NuPAGE™ MOPS SDS Running Buffer (20X) **Invitrogen - Thermo Fisher Catalog #NP000102**

12. 1 X Towbin transfer buffer: 25 undetermined Tris, 192 undetermined Glycine, 20% methanol

13. 1X Tris Buffered-Saline (TBS): 500 undetermined Tris, 150 undetermined sodium chloride, pH 7.6, at 25 °C .

14. 1X Tris-Buffered Saline, 0.1% Tween® 20 Detergent (TBST)

15. 5 % non-fat milk in TBST

16. 5%

Bovine Serum Albumin Fraction V **Merck MilliporeSigma (Sigma-Aldrich) Catalog #10735094001**

17. **Primary antibodies:**

Phospho-Ubiquitin (Ser65) (E2J6T) Rabbit mAb **Cell Signaling Technology Catalog #62802**

Purified anti-Ubiquitin Antibody **BioLegend Catalog #646302**

Anti-Parkin phospho-Ser65 Rabbit mAb by Epitomics in collaboration with the Michael J Fox Foundation for Research,

Parkin Antibody (PRK8) **Santa Cruz Biotechnology Catalog #32282**

GAPDH Antibody (6C5) **Santa Cruz Biotechnology Catalog #32233**

18. Secondary Antibodies:

Goat anti-Rabbit IgG (H L) Secondary Antibody HRP **Invitrogen - Thermo Fisher Catalog #31460**

Rabbit anti-Mouse IgG (H L) Secondary Antibody HRP **Invitrogen - Thermo Fisher Catalog #31450**

19. ECL™ Western Blotting Reagents (Merck, Cytiva, #RPN2106)

ECL™ Western Blotting Reagents **Merck Catalog #RPN2106**

20. Amersham Hyperfilm ECL (Merck, Cytiva, #28906837)

Hyperfilm™ ECL™ **Merck Catalog #28906837**

STOCK SOLUTION PREPARATION:

- **Poly-L-lysine solution:** Dissolve [M] 10 mg/mL of poly-L-lysine in sterile water; filter, aliquot and store at -20 °C . The solution is stable for 2–3 months.
- **DNaseI:** Dissolve [M] 100 mg/mL (wt/vol) DNaseI in sterile double-distilled water; filter, aliquot and store at -20 °C . The solution is stable for 2–3 months.
- **Antimycin A:** Prepare 50 undetermined of Antimycin A in DMSO; aliquot and store at -20 °C .
- **Oligomycin A:** Prepare 10 undetermined of Antimycin A in DMSO; aliquot and store at -20 °C .

EQUIPMENT

- Dumont #5 Forceps Biologie Inox (Fine Science Tool #11252-20)

Equipment	
Dumont #5 Forceps	NAME
Forceps	TYPE
Fine Science Tools	BRAND
11252-20	SKU
https://www.finescience.com/en-US/Search?searchtext=11252-20	LINK

- Dumont #5XL Forceps Standard Inox (Fine Science Tool #11253-10)

Equipment	
Dumont #5XL Forceps	NAME
Forceps	TYPE
Fine Science Tools	BRAND
11253-10	SKU
https://www.finescience.com/en-US/Search?searchtext=11253-10	LINK

- Dumont #7 Fine Forceps Biologie Inox (Fine Science Tool #11274-20)

Equipment	
Dumont #7 - Fine Forceps	NAME
Forceps	TYPE
Fine Science Tools	BRAND
11274-20	SKU
https://www.finescience.com/en-US/Search?searchtext=11274-20	LINK

- Dumont #5 45 Forceps Standard Dumoxel (Fine Science Tool #11251-35)

Equipment	
Dumont #5/45 Forceps	NAME
Forceps	TYPE
Fine Science Tools	BRAND
11251-35	SKU
https://www.finescience.com/en-US/Search?searchtext=11251-35	LINK

- Fine Scissors ToughCut Straight 9cm (Fine Science Tool #14058-09)

Equipment	
Fine Scissors - ToughCut®	NAME
Scissor	TYPE
Fine Science Tools	BRAND
14058-09	SKU
https://www.finescience.com/en-US/Search?searchtext=14058-09	LINK

- Dissecting microscope Nikon (P-PS32 Plain Stand) with fiber light illumination unit
- Cell Counter-DeNovix CellDrop™
- 37 °C water bath
- Laminar flow cell culture hood
- Cell culture incubator 5% CO₂, 95% humidity HERAcell®CO₂ incubator (150 L)
- Microcentrifuges, Micro Star 17R (VWR #521-1647)

Equipment	
Microcentrifuges, ventilated/refrigerated, Micro Star 17 / 17R	NAME
Microcentrifuges	TYPE
VWR	BRAND
521-1647	SKU
https://uk.vwr.com/store/product?keyword=521-1647	LINK

- Probe sonicator, Branson Digital Sonifier.
- XCell SureLock™ 4Midi-Cell running tank (Invitrogen™ #WR0100)

Equipment	
XCell4 SureLock™ Midi-Cell	NAME
Midi-Cell running tank	TYPE
Invitrogen	BRAND
WR0100	SKU
https://www.thermofisher.com/order/catalog/product/WR0100?SID=srch-srp-WR0100#/WR0100?SID=srch-srp-WR0100	LINK

- XCell SureLock™ Mini-Cell running tank (Invitrogen™ #EI0001)

Equipment	
XCell SureLock Mini-Cell Electrophoresis System	NAME
Electrophoresis System	TYPE
Invitrogen	BRAND
EI0001	SKU

- Mini Trans-Blot®Cell transfer tank (BIORAD # 1703930)

Equipment	
Mini Trans-Blot Electrophoretic Transfer Cell	NAME
Electrophoresis System	TYPE
Mini Trans-Blot	BRAND
1703930	SKU
https://www.bio-rad.com/en-uk/sku/1703930-mini-trans-blot-electrophoretic-transfer-cell?ID=1703930	LINK

- Trans-Blot®Cell transfer tank (BIORAD # 1703939)

Equipment	
Trans-Blot Cell With Plate Electrodes and Super Cooling Coil	NAME
Trans-Blot	BRAND
1703939	SKU
https://www.bio-rad.com/en-uk/sku/1703939-trans-blot-cell-with-plate-electrodes-super-cooling-coil?ID=1703939	LINK

- ChemiDoc MP Imaging System (BIORAD)
- ECOMAX™ X-ray Processor

CONSUMABLES

- Cell culture multidishes, 6 well (Thermo Scientific™ #140675)

Equipment	
Nunc 6-Well Plate, Round	NAME
cell culture plate	TYPE
Thermo Fisher Scientific	BRAND
140675	SKU
https://www.thermofisher.com/order/catalog/product/140675	LINK
Nunc 6-Well Plate, Round	SPECIFICATIONS

- Cell strainer 40µM (Greiner Bio-one #5420400)

Equipment	
EASYSTRAINER 40 µM, FOR 50 ML TUBES, FOR TUBES 227XXX/210XXX, GREEN, STERILE, SINGLE PACKED	NAME
Cell strainer	TYPE
Greiner Bio-one	BRAND
542040	SKU
https://shop.gbo.com/en/england/products/bioscience/cell-culture-products/easystainer-cell-strainer/542040.html?_ga=2.140829938.1748636939.1614536280-782045221.1614536280	LINK

- Stericups 0.22um, 250 mL and 500 mL (Merck #SCGPU02RE, #SCGPU05RE)

Equipment	
Stericup-GP Sterile Vacuum Filtration System	NAME
Sterile Vacuum Filtration System	TYPE
Merck	BRAND
SCGPU05RE	SKU
https://www.merckmillipore.com/GB/en/product/Stericup-GP-Sterile-Vacuum-Filtration-System-this-item-has-been-replaced-with-S2GPU05RE,MM_NF-SCGPU05RE	LINK

Equipment	
Stericup-GP Sterile Vacuum Filtration System	NAME
Sterile Vacuum Filtration System	TYPE
Merck	BRAND
SCGPU02RE	SKU
https://www.emdmillipore.com/US/en/product/Stericup-GP-Sterile-Vacuum-Filtration-System,MM_NF-SCGPU02RE?bd=1	LINK

- 50mL Stripette® Serological Pipets (Corning #4490)

Equipment	
Stripette™ Serological Pipets	NAME
Serological Pipet	TYPE
Stripette	BRAND
4490	SKU
https://www.corning.com/worldwide/en/search.html?productsSearchState=&resourcesSearchState=&relatedContentSearchState=&initialResultType=products&searchText=4490&search-initialcatalog=Corporate%20Communications&search-region=US	LINK

- 25mL Stripette® Serological Pipets (Corning #4489)

Equipment	
25 mL Stripette™ Serological Pipets, Polystyrene, Individually Paper/Plastic Wrapped, Sterile, 25/Bag, 200/Case	NAME
Serological Pipet	TYPE
Stripette	BRAND
4489	SKU
https://ecatalog.corning.com/life-sciences/b2c/US/en/Liquid-Handling/Pipets/Serological-Pipets/Stripette%E2%84%A2-Serological-Pipets/p/4489	LINK

- 10mL Stripette® Serological Pipets (Corning #4488)

Equipment	
Stripette™ Serological Pipets	NAME
Serological Pipet	TYPE
Stripette	BRAND
4488	SKU
https://ecatalog.corning.com/life-sciences/b2c/US/en/Liquid-Handling/Pipets/Serological-Pipets/Stripette%E2%84%A2-Serological-Pipets/p/stripetteSerologicalPipets	LINK

- 5mL Stripette® Serological Pipets (Corning #4487)

Equipment	
5 mL Stripette™ Serological Pipets, Polystyrene, Individually Paper/Plastic Wrapped, Sterile, 50/Bag, 200/Case	NAME
Serological Pipet	TYPE
Stripette	BRAND
4487	SKU
https://ecatalog.corning.com/life-sciences/b2c/US/en/Liquid-Handling/Pipets/Serological-Pipets/Stripette%E2%84%A2-Serological-Pipets/p/4487	LINK

- 15ml CELLSTAR® tubes (Greiner bio-one. Catalog# 188271)

Equipment	
15 ml CELLSTAR® Polypropylene Tube	NAME
Polypropylene Tube	TYPE
CELLSTAR	BRAND
188271	SKU
https://shop.gbo.com/en/england/products/bioscience/tubes-beakers/tubes/15ml-cellstar-polypropylene-tube/188271.html?_ga=2.62696428.1748636939.1614536280-782045221.1614536280	LINK

- 50ml CELLSTAR® tubes (Greiner bio-one. Catalog# 227261)

Equipment	
50 ml CELLSTAR® Polypropylene Tube	NAME
Polypropylene Tube	TYPE
CELLSTAR	BRAND
227261	SKU
https://shop.gbo.com/en/england/products/bioscience/tubes-beakers/tubes/50ml-cellstar-polypropylene-tube/227261.html?_ga=2.128248780.1748636939.1614536280-782045221.1614536280	LINK

- Standard 1ml and 200µl Pipette tips (Greiner bio-one # 686271, #685261)

Equipment	
100 - 200 µl Pipette Tips	NAME
Pipette Tip	TYPE
Greiner bio-one	BRAND
685261	SKU
https://shop.gbo.com/en/england/products/bioscience/liquid-handling/pipette-tips/100-200ul-pipette-tips/685261.html	LINK

Equipment	
1000 µl Pipette Tips	NAME
Pipette Tip	TYPE
Greiner bio-one	BRAND
686271	SKU
https://shop.gbo.com/en/england/products/bioscience/liquid-handling/pipette-tips/100ul-pipette-tips/686271.html	LINK

- Syringe filter (0.22µm. Sartorius, Item # ST16541-Q)

Equipment	
Minisart® Syringe Filter, Polyethersulfone (PES), Pore Size 0.22 µm, Non-Sterile, Female Luer Lock, Male Luer Slip, Pack Size 500	NAME
Syringe Filter	TYPE
Sartorius	BRAND
ST16541-Q	SKU
https://www.sartorius.com/shop/ww/en/usd/applications-laboratory-filtration-sterile-filtration/minisart-syringe-filter%2c-polyethersulfone-%28pes%29%2c-pore-size-0-22-%c2%b5m%2c-non-sterile%2c-female-luer-lock%2c-male-luer-slip%2c-pack-size-500/p/16541---	LINK

- Syringes (50ml) (Terumo™ # 8SS50L1)

Equipment	
Terumo™ 3-Part 50mL Luer Lock Syringes	NAME
Luer Lock Syringe	TYPE
Terumo	BRAND
8SS50L1	SKU
https://www.fishersci.co.uk/shop/products/3-part-50ml-luer-lock-syringes/15349067	LINK

- 1.5ml Eppendorf tubes (Eppendorf™ # 0030120086)

Equipment	
Eppendorf® Safe-Lock micro test tubes	NAME
Micro test tube	TYPE
Eppendorf	BRAND
0030120086	SKU
https://www.sigmaaldrich.com/catalog/product/sigma/ep0030120086?lang=en&region=US	LINK

- Disposable Cell Lifter (FisherBrand #08100240)

Equipment	
Fisherbrand™ Cell Lifters	NAME
Cell Lifter	TYPE
Fisherbrand	BRAND
08100240	SKU
https://www.fishersci.com/shop/products/fisherbrand-cell-lifters/08100240#?keyword=08100240	LINK

Coating of multiwell dishes with poly-L-lysine

1

Note

◇TIMING 🕒 00:30:00 , 1 d before culture in this section.

In a sterile laminar hood, prepare poly-L-lysine [M] 1 mg/mL in sterile from [M] 10 mg/mL stock solution.

2

Add enough poly-L-lysine solution to cover the bottom of the well, ensure that the volume of poly-L-lysine covers the bottom of the dish entirely, usually 🧪 1 mL for 1 single well.

3

Wrap the dish in aluminum foil to prevent evaporation and leave it 🕒 Overnight at 🌡 Room temperature .

Note

It is important to make sure that the poly-L-lysine does not dry out during incubation. If you are working in the laminar hood, be sure to turn the blower off during the incubation

Washing of dishes after coating

4

Note

◇TIMING 🕒 01:00:00 , day of culture in this section.

Aspirate the poly-L-lysine carefully.

5

Add 🧪 1 mL of sterile water into each well and aspirate; repeat this step three times.

- 6 Aspirate water and leave dishes under the hood until completely dry.

Dissection of cortex from E16.5 mouse embryos

7

Note

⌚TIMING ⌚ 01:00:00 , ⌚ 00:02:00 – ⌚ 00:05:00 for each embryo in this section.

Use sterilized instruments by autoclave or washing them with 70% (vol/vol) ethanol.

Note

Dry thoroughly if ethanol is used.

8

Prepare 60-mm dishes with dissection medium. If you are culturing from individual embryos, prepare 15 mL tubes with 1 mL of dissection medium. If you are culturing pooled embryos, prepare 15 mL tubes with 5 mL of dissection medium.

9

Euthanize the embryos pup by decapitation and separate the head from the body.

10

Place the head on a dish with dissection medium and hold down the sides with forceps.

11

Under a dissecting microscope, dissect the skin on the top of the head and hold down the skin on either side with the forceps.

- 12** Cut open the skull by making an incision at the base of the brain. Separate the two halves of the skull and remove carefully.

Note

Take care to not cut through the brain tissue when removing the skull bone

- 13** By using forceps, pinch off the brain from the base and peel off the meninges carefully, ideally as a single piece. Check for the remaining pieces of meninges and remove them completely.

Note

The meninges can be sticky and difficult to remove, it is important to ensure that the meninges are completely removed, so that they do not contribute any non-neuronal cells to the culture.

- 14** Separate the two halves of the brain by making a sagittal cut along the midline.

- 15** Orient the tissue so that the hippocampus is on the top. The hippocampus can be identified by its C-shaped structure and opacity, which differ from the neighboring cortical tissue. Cut away hippocampus and flat the cortex to expose the striatum. Using an angled forceps scoop out the striatum and cortex can also be dissected out and processed for cortical cultures.

Note

It is important to ensure that the hippocampus and striatum are completely removed, so that they do not affect cortical distribution of neurons and contribute to an increase of glia cells.

- 16** To culture neurons from individual embryo, put each brain in a separate 15 mL tube containing 1 mL of dissection medium. For pooled cultures, put cortices from two embryos into one 15 mL tube containing 5 mL of dissection medium.

Note

Ensure that the cortices are submerged in the medium and do not let them dry out at any point. It is important that the dissection be done as quickly as possible to ensure cell viability and health.

- 17 For individual cultures, collect a piece of tail for genotyping.

Cell dissociation and plating

42m

18

Note

⚡TIMING ~ ⌚ 01:00:00 | ⌚ 01:30:00 in this section.

Prepare digestion medium by adding 125 μL of DNase I (stock solution 10 mg/mL) to 10 mL of Trypsin 0,05%.

19

Add 1:1 digestion medium to dissection medium containing the cortices, for individual mouse culture add 1 mL of digestion media, for pooled mouse culture add 5 mL of digestion media.

Note

Note. It is common to use HBSS Ca^{2+} and Mg^{2+} free buffer. In our hands, we have noticed that using HBSS plus Ca^{2+} and Mg^{2+} ensure a milder but optimal concentration of trypsin that allows to reduce excessive number of dead cells and higher yield of cells. At the same time, it stimulates DNase I activity.

20

Incubate at 37 °C in a water bath for 00:30:00 .

30m

Note

It is important to ensure that this incubation does not proceed for longer than 00:30:00

21

Inactivate trypsin digestion by adding dissociation medium, 2 mL for individual mouse culture or 5 mL for pooled mouse culture.

22

Centrifuge at 1200 rpm for 00:05:00 .

5m

23

Resuspend cortices in 2 mL (for individual embryos) or 4 mL (for pooled embryos) of dissociation medium.

24

Carefully triturate the tissue 20 times to dissociate the cells gently and obtain a homogenous cell suspension, by using a p1000 pipette.

Note

The trituration of cells should be done slowly and carefully to minimize damage to cells. It is best to avoid any bubbling during the procedure.

25

Remove any chunks of tissue by using a cell strainer and distribute drop by drop the cell suspension.

26

Centrifuge at 700 rpm for 00:07:00 to pellet down the cells and resuspend them in the culturing medium.

7m

Note

Note: It is important to remove FBS from the medium to reduce the proliferation of glial cells.

27 Resuspend the dissociated cells in 2 mL (for individual embryos) or 4 mL (for pooled embryos) of culturing medium.

28 Take a 15 μ L aliquot, add 1:1 ratio Trypan Blu and determine the density of cells and cell viability to the cell counter. Typical yields are $\sim 2 \times 10^6$ /mL and viability >80 %.

29 5.0×10^5 cells/well plates are plated out on 6-well multidishes, containing 2 mL of pre-warmed culturing media.

Maintenance of neurons

30

Note

⌚TIMING 72:00:00 Up to 504:00:00 in this section.

Every 5 days aspirate 1/3 of the media from each well and replace it with fresh culturing medium warmed to 37 °C . These neurons can be maintained in culture for up to DIV 28 and be used anytime during this period (DIV0-28). The culture can be used for examining PINK1 activation at 21DIV, since at this stage they express functional activity of Parkin and PINK1.

Note

It is important to not replace the entire medium, neurons secrete factors that promote growth and survival.