ioGlutamatergic Neurons

TDP-43 • M337V/WT

User Manual

ioGlutamatergic Neurons
TDP-43$^{M337V/WT}$
Early Access Product
Catalogue No: ioEA1006

User Manual
Document NPI-0032 UM V-01

For research use only

bit.bio
The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
www.bit.bio

Customer support:
technical@bit.bio
orders@bit.bio

bit.bio
THE CELL CODING COMPANY

bit.bio
The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
www.bit.bio

Customer support:
technical@bit.bio
orders@bit.bio

Before starting

- bit.bio recommends reading this protocol in its entirety before the revival of the ioGlutamatergic Neurons TDP-43$^{M337V/WT}$ cells.
- Transfer the vials of ioGlutamatergic Neurons TDP-43$^{M337V/WT}$ to liquid nitrogen or to -150°C immediately after receipt.
- Before the revival of ioGlutamatergic Neurons TDP-43$^{M337V/WT}$, prepare the tissue culture plates or flasks coated with PDL-Geltrex (Appendix 5.5).
- The recommended reagents for the revival and maintenance of ioGlutamatergic Neurons TDP-43$^{M337V/WT}$ can be found in Appendix 5.3.

Important information:

- ⚠This product, ioGlutamatergic Neurons TDP-43$^{M337V/WT}$ is referred to as ioGlutamatergic Neurons TARDBP$^{M337V/WT}$ on the vial label for lot numbers:
  220614W1 and 220704W1 for ioEA1006S
  220624W1 and 220624W2 for ioEA1006L
- This protocol has been designed for the ioGlutamatergic Neurons TDP-43$^{M337V/WT}$ disease model and its isogenic wild-type control ioGlutamatergic Neurons (catalogue number: io1001).
- ioGlutamatergic Neurons TDP-43$^{M337V/WT}$ are for Research and Development use only. User agrees to use the Product in compliance with all applicable statutes and regulations, but not to use the Product for any administration or application to humans. Moreover, User agrees not to use the Product in human subjects for human clinical use for therapeutic, diagnostic or prophylactic purposes, or in animals for veterinary use for therapeutic, diagnostic or prophylactic purposes, including but not limited to clinical applications, cell therapy, transplantation, and/or regenerative medicine without an appropriate license.
- ioGlutamatergic Neurons TDP-43$^{M337V/WT}$ are cryopreserved in a Cryopreservation Medium containing 10% DMSO: the safety data sheet (SDS) of dimethyl sulfoxide (DMSO) is available on request.
- ioGlutamatergic Neurons TDP-43$^{M337V/WT}$ should only be used by personnel qualified in handling human biological materials following local health and safety regulations.

bit.bio
The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
www.bit.bio

Customer support:
technical@bit.bio
orders@bit.bio

1. Overview of ioGlutamatergic Neurons TDP-43$^{M337V/WT}$

bit.bio glutamatergic neurons, referred to as ioGlutamatergic Neurons TDP-43$^{M337V/WT}$ throughout this user manual, rapidly mature into functional glutamatergic neurons after revival in the recommended medium. They are delivered in a convenient cryopreserved format and provide a homogeneous and reproducible model for human excitatory neurons.

ioGlutamatergic Neurons TDP-43$^{M337V/WT}$ cultures consist mainly of glutamatergic neurons characterised by the expression of the glutamate transporter genes VGLUT1 and VGLUT2. The minor remaining fraction of the neuronal population express marker genes of cholinergic neurons.

A bulk RNA-sequencing analysis shows that ioGlutamatergic Neurons TDP-43$^{M337V/WT}$ have a rostral CNS identity.

The protocol for the generation of ioGlutamatergic Neurons TDP-43$^{M337V/WT}$ is a three-phase process. Phase 0 - Induction is carried out at bit.bio before distribution (Fig 1A).

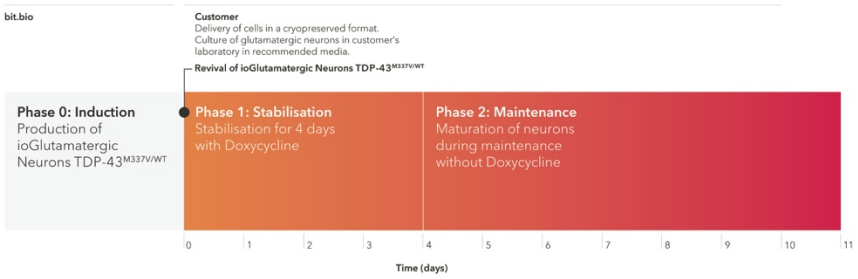

Figure 1A
Schematic representation of the three-phase protocol to produce and culture ioGlutamatergic Neurons TDP-43$^{M337V/WT}$.

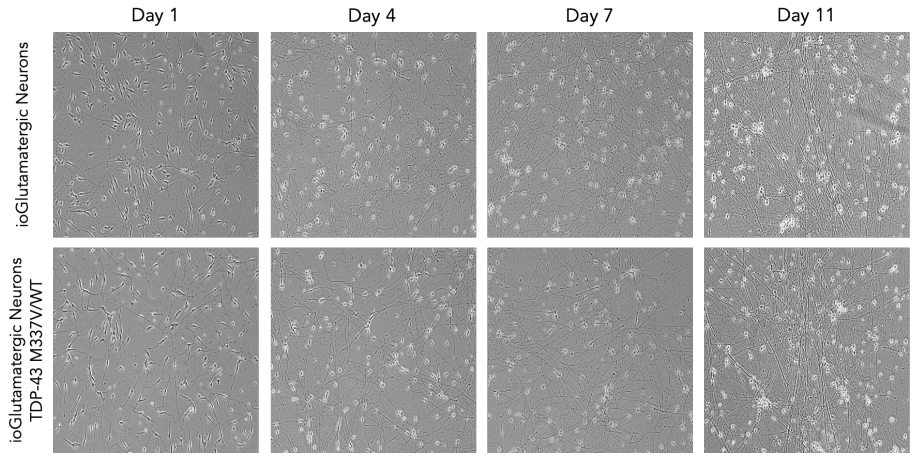

Figure 1B
Brightfield images of ioGlutamatergic Neurons TDP-43$^{M337V/WT}$ after revival over the course of the first 11 days of culture (Day 1 to 11 post-thawing; 100X magnification)

bit.bio	**Phase 0 – Induction (day -3 to 0):** Human iPSCs are exposed to an induction protocol. This leads to irreversible loss of pluripotency, and the synchronised homogenous production of glutamatergic neurons. The ioGlutamatergic Neurons TDP-43$^{M337V/WT}$ are subsequently cryopreserved for distribution.
User	**Phase 1 – Stabilization (day 0 to 4):** The ioGlutamatergic Neurons TDP-43$^{M337V/WT}$ are revived at the user's laboratory using the recommended medium supplemented with doxycycline (96h) and DAPT (day 2-4) for sustained induction. **Phase 2 – Maintenance (day 4 onwards):** Depending on assay requirements, the ioGlutamatergic Neurons TDP-43$^{M337V/WT}$ can be used over different lengths of time in the maintenance medium. Note: ioGlutamatergic Neurons (cat no: io1001), the isogenic wild-type control for ioEA1006, have been maintained up to 28 days in the above conditions without impairment to neuronal health, function and culture attachment.

Table 1
Description of the three-phase protocol for the production and culture of ioGlutamatergic Neurons TDP-43$^{M337V/WT}$.

bit.bio
The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
www.bit.bio

Customer support:
technical@bit.bio
orders@bit.bio

bit.bio
The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
www.bit.bio

Customer support:
technical@bit.bio
orders@bit.bio

2. Culture of ioGlutamatergic Neurons TDP-43$^{M337V/WT}$

Before starting:

- Each ioGlutamatergic Neurons TDP-43$^{M337V/WT}$ vial contains either ≥1.0x10^6 (Small vial) or ≥5.0x10^6 (Large vial) viable cells. bit.bio recommends a minimum seeding density of 30,000 cells/cm^2.
- Prepare enough tissue culture vessels with PDL-Geltrex coating prior to reviving the cryovial(s) (Appendix 5.5).
- Warm-up the water bath to 37°C.
- Warm-up **basal glutamatergic neuron (b:GN) medium** to 37°C (Appendix 5.4).
- Prepare the **complete glutamatergic neuron (comp:GN) medium** supplemented by **1µg/mL doxycycline (D) (comp:GN+D)** for revival (Appendix 5.4).

Revival and culture procedures:

2.1 – Cell thawing

1. Remove the cryovial(s) from dry ice and immediately immerse into a 37°C water bath (or similar) while maintaining a constant gentle agitation.
2. Remove the cryovial(s) from the water bath when only a very small fraction of ice is left visible (this should take approximately 2 minutes).
3. Spray the cryovial(s) with 70% ethanol and take it to a biological safety cabinet.
4. Transfer the cells from each vial into an empty 15mL tube and add 1mL of **b:GN medium** in a dropwise manner, agitating the tube occasionally. ⚠Cryopreservation medium contains DMSO: minimise the time between thawing and centrifugation of cells and ensure the suspension is thoroughly mixed with **b:GN medium** before centrifugation.
5. Add a further 3mL of **b:GN medium** per tube in a dropwise manner, agitating the tube occasionally.
6. Carefully wash the cryovial(s) with 1mL of **b:GN medium** and gently add it to the tube(s).
7. Centrifuge the cells at 200 g for 3 min at room temperature.
8. Carefully remove the supernatant by aspiration.
9. Add 1mL of **comp:GN+D medium** to the cell pellet and gently resuspend the cells by pipetting up-and-down with a 1mL micropipette.
10. Count the cells including a cell viability marker. The typical recovery from one cryovial is ≥1.0x10^6 viable cells (Small vial) or ≥5.0x10^6 viable cells (Large vial).

bit.bio
The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
www.bit.bio

Customer support:
technical@bit.bio
orders@bit.bio

2.2 – Cell seeding (Day 0)

11. Dilute the cell suspension to the required cell concentration (table below for reference) using **comp:GN+D medium** to achieve the optimal seeding density for your desired experimental conditions (Section 3 for 96 and 384 well plate cultures). A seeding density of 30,000 cells/cm^2 is routinely used at bit.bio.

Plate format	Surface (cm^2)	mL/well	Cells/well	Cells/mL
6 well	9.5	2.5	285,000	114,000
12 well	3.8	1	114,000	114,000
24 well	1.9	0.5	57,000	114,000
48 well	0.95	0.25	28,500	114,000

12. Aspirate the Geltrex coating solution from the culture vessel(s).
13. Directly add the required volume of cell suspension to the culture vessel(s), occasionally agitating the tube to ensure consistent cell distribution.
14. Immediately transfer the culture vessel(s) to a standard normoxic tissue culture humidified incubator at 37°C, 5% CO_2.
15. To ensure an even cell distribution, gently cross-shake the plate once on the incubator shelf (back and forth, side to side, 2-3 times).

2.3 – Cell stabilization (Day 0 to 4) and maintenance (Day 4 onwards)

16. Day 2: 48h post thawing, completely replace the culture medium with fresh pre-warmed **comp:GN+D medium supplemented with 10µM of DAPT (comp:GN+D+DAPT)**.
    ⚠Culture of ioGlutamatergic Neurons TDP-43$^{M337V/WT}$ should be carried out with special care as neuronal cells are prone to mechanical stress which may cause detachment.
    ⚠ Medium aspiration should be halted with ~10% of the medium left in the well.
    ⚠Medium addition should be performed slowly and on the side of the well, using micropipettes instead of serological pipettes.
17. Day 4: 96h post thawing, completely replace the culture medium with fresh pre-warmed **comp:GN medium** (no doxycycline).
18. Day 6 onwards: for optimal glutamatergic neuron maintenance, bit.bio recommends half-medium change every 48h, i.e., replacing 50% of the medium with fresh **comp:GN medium** (no doxycycline).

3. Culture of ioGlutamatergic Neurons TDP-43$^{M337V/WT}$ in 96 or 384 well plates

The following protocol has been optimized for the revival and culture of ioGlutamatergic Neurons TDP-43$^{M337V/WT}$ directly into 96 or 384 well plates. Note that the optimal cell seeding density will depend on the specific experimental aims defined by the user.

1. Pre-coat the required wells with PDL-Geltrex (Appendix 5.5).
2. Thaw the cells as per the standard protocol described in Section 2.1.
3. After the cell counting, adjust the cell suspension concentration using **comp:GN+D medium** to achieve the targeted seeding density (see table below for reference):

Seeding density (cells/cm²)	384well (0.056cm², 30µL)		96well (0.32cm², 100µL)	
	cells/well	cells/mL	cells/well	cells/mL
30,000	1,680	56,000	9,600	96,000
40,000	2,240	74,666	12,800	128,000
50,000	2,800	93,333	16,000	160,000

Recommendation: bit.bio recommends a seeding density between 30,000 to 50,000 cells/cm^2 in a final volume of 30µL per 384 well or 100µL per 96 well.
Note: when calculating the total volume of cell suspension required, consider preparing 10% more to accommodate for volume losses during cell handling.

4. Aspirate the Geltrex coating medium from the plate.
5. Pour the adjusted seeding cell suspension into a reservoir suitable for multichannel pipettes, occasionally using a pipette to mix the cell suspension for even cell distribution.
6. Using a multichannel pipette, add 30µL or 100µL of the cell suspension into the wells of the 384 or 96 well plate, respectively.
7. Transfer the culture plate into the standard normoxic tissue culture humidified incubator at 37°C, 5% CO_2.
8. Day 2: 48h post thawing, completely replace the culture medium with fresh pre-warmed **comp:GN+D+DAPT medium** leaving around ~10% of the medium in the well. Use the same volumes as were used for seeding. Be gentle during the media replacement to avoid cell detachment.
9. Day 4: 96h post thawing, completely replace the culture medium with double the original volume of fresh pre-warmed **comp:GN medium** (no doxycycline). (200µL and 60µL into the wells of a 96 and 384 well plate, respectively).
10. Day 6 onwards: for optimal glutamatergic neuron maintenance, bit.bio recommends a half-medium change regime every 48h, i.e., replacing 50% of the medium with fresh **comp:GN medium** (no doxycycline).

bit.bio
The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
www.bit.bio

Customer support:
technical@bit.bio
orders@bit.bio

bit.bio
The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
www.bit.bio

Customer support:
technical@bit.bio
orders@bit.bio

4. Co-culture of ioGlutamatergic Neurons with Astrocytes

This protocol has been designed for ioGlutamatergic Neurons (cat no: io1001), the isogenic wild-type control for ioEA1006.

Possible application: to determine the electrophysiological properties of neurons.

Before starting:

- Prepare enough tissue culture vessels with PDL-Geltrex coating prior to reviving the cryovial(s) (Appendix 5.5). bit.bio recommends a 1:1 co-culture ratio of ioGlutamatergic Neurons TDP-43$^{M337V/WT}$ and astrocytes, and a seeding density of 30,000 cells/cm^2 of each cell type (total of 60,000 cells/cm^2).
  ⚠If these cells are being used for a functional experiment, e.g. MEA, the ideal seeding density may be significantly different. Please refer to the assay manufacturer's guidelines for their recommendations.
- Warm-up the water bath to 37°C.
- Warm-up **basal glutamatergic neuron (b:GN) medium** to 37°C (Appendix 5.4).
- Prepare the **complete glutamatergic neuron (comp:GN) medium** supplemented by **1µg/mL doxycycline (comp:GN+D)** for revival (Appendix 5.4).
- Prepare astrocytes for seeding according to manufacturer/author's protocol, in parallel to section 4.1.2 of this protocol. If not possible, prepare astrocytes first and keep the cell suspension in a standard normoxic tissue culture humidified incubator at 37°C, 5% CO_2, occasionally shaking the cell suspension. Make sure the cap of the vial or tube is not fully closed. Proceed immediately to section 4.1.3.

Revival and culture procedures:

4.1 – Cell thawing and seeding (Day 0)

1. Pre-coat the required wells with PDL-Geltrex (Appendix 5.5).
2. Thaw the cells as per the standard protocol described in Section 2.1.
3. Resuspend astrocytes and ioGlutamatergic Neurons TDP-43$^{M337V/WT}$ at the appropriate cell concentration in **comp:GN+D medium** to achieve the required seeding density for your desired experimental conditions. A minimum seeding density of 30,000 cells/cm^2 of each cell type is routinely used at bit.bio.
4. Mix both cell suspensions to achieve a homogenous 1:1 ratio mixed-cell suspension.
5. Aspirate the Geltrex coating solution from the culture vessel(s).
6. Directly add the required volume of cell suspension to the culture vessel(s).
7. Immediately transfer the culture vessel(s) to a standard normoxic tissue culture humidified incubator at 37°C, 5% CO_2.

8. To ensure an even cell distribution, gently cross-shake the plate once on the incubator shelf (back and forth, side to side, 2-3 times).

4.2 – Cell stabilization (Day 0 to 4) and maintenance (Day 4 onwards)

Day 2: 48h post thawing, completely replace the culture medium with fresh pre-warmed **comp:GN+D+DAPT medium**.
⚠Culture of ioGlutamatergic Neurons TDP-43$^{M337V/WT}$ should be carried out with special care as neuronal cells are prone to mechanical stress which may cause detachment.
⚠ Medium aspiration should be halted with ~10% of the medium left in the well.
⚠Medium addition should be performed slowly and on the side of the well, using micropipettes instead of serological pipettes.

9. Day 4: 96h post thawing, completely replace the culture medium with fresh pre-warmed **comp:GN medium** (no doxycycline).

Note: a single addition of 2µM Cytarabine (ara-C) to arrest further astrocyte growth is recommended at this stage.

10. Day 6 onwards: for optimal astrocyte-glutamatergic neuron co-culture maintenance, bit.bio recommends a half-medium change regime every 48h, i.e., replacing 50% of the medium with fresh **comp:GN medium** (no doxycycline).

Note: bit.bio co-cultures have been carried out with primary astrocytes derived from P0-P2 neonatal Sprague Dawley rats. These astrocytes have demonstrated good long-term survival and functionality in comp:GN medium, without the need for foetal bovine serum (FBS) in the medium. Co-culture of glutamatergic neurons with rat astrocytes have demonstrated good survival up to 100 days post-thawing, with first instance of electrophysiological activity at 8 days (+/- 2 days) post-thawing. If using astrocytes from other sources, such as human PSC derived astrocytes, comp:GN may need to be supplemented with growth factors recommended in manufacturer/author's protocol.

bit.bio
The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
www.bit.bio

Customer support:
technical@bit.bio
orders@bit.bio

bit.bio
The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
www.bit.bio

Customer support:
technical@bit.bio
orders@bit.bio

5. Appendices

5.1 – Equipment required

- Biological safety cabinet
- Normoxic cell culture incubator (37°C, 5% CO_2)
- 37°C water bath or equivalent
- Haemocytometer or calibrated automatic cell counter
- Liquid nitrogen storage unit
- Standard tissue culture wares (pipettes, tips, culture plates)
- Bench top centrifuge

5.2 – Recommended reagents

Reagent	Supplier	Cat. number	Storage
Geltrex (Reduced GF)	ThermoFisher	A1413202	-20°C to -80°C
PDL-hydrobromide	Sigma	P6407	-20°C to -80°C
Borate buffer (20x)	ThermoFisher	28341	Room temperature
Sterile water	Sigma	W3500	Room temperature
DMEM/F-12	ThermoFisher	11330032	2°C to 8°C
Neurobasal	ThermoFisher	21103049	2°C to 8°C
B27	ThermoFisher	17504044	-20°C to -80°C
Glutamax	ThermoFisher	35050061	2°C to 8°C
2-Mercaptoethanol	ThermoFisher	31350010	2°C to 8°C
NT3	R&D	267-N3-005	-20°C to -80°C
BDNF	R&D	248-BDB-005	-20°C to -80°C
DAPT	Biotechne	2634	2°C to 8°C
Doxycycline	Sigma	D9891	2°C to 8°C
Bovine Serum Albumin	Sigma	A7906	2°C to 8°C
Cytarabine (ara-C)	Sigma	C1768	2°C to 8°C

5.3 – Preparation of stock solutions

Note: to avoid freeze-thaw cycles, aliquot the stock solutions as appropriate for future use.

Reagent	Stock solution	Working concentration
NT3	50µg/mL (5000X solution) *To prepare, reconstitute 25µg in 500µL of PBS containing 0.1% BSA*	10ng/mL *0.2µL of stock solution per 1mL of medium*
BDNF	10µg/mL (2000X solution) *To prepare, reconstitute 5µg in 500µL of PBS containing 0.1% BSA*	5ng/mL *0.5µl of stock solution per 1mL of medium*
DAPT	20mM (2000X solution) *To prepare, reconstitute 10mg in 1156µL of DMSO according to the manufacture's protocol*	10µM *0.5µL of stock solution per 1mL of medium*
Doxycycline (DOX)	2mg/mL (2000X solution): *To prepare, reconstitute 20mg in 10mL of H_2O*	1µg/mL *0.5µL of stock solution per 1mL of medium*
Cytarabine (ara-C)	20mM (10,000X solution) *To prepare, reconstitute 10mg in 2mL of water*	2µM *0.1µL of stock solution per 1mL of medium*

bit.bio
The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
www.bit.bio

Customer support:
technical@bit.bio
orders@bit.bio

5.4 – Preparation of glutamatergic neuron medium

- **b:GN: basal** glutamatergic neuron medium

Reagent/ Media	For 200mL	For 500mL
Neurobasal	200mL	500mL
Glutamax (100X)	2mL	5mL
2-Mercaptoethanol (final conc. 25µM)	100µL	250µL

Note: The basal medium is stable for 3 weeks at 4°C.

- **comp:GN: complete** glutamatergic neuron medium

Reagent/ Media	For 50mL	For 200mL
b:GN	50mL	200mL
B27	1mL	4mL
NT3 (final conc. 10ng/mL)	10µL	40µL
BDNF (final conc. 5ng/mL)	25µL	100µL

Note: The complete medium is better prepared fresh before each feeding. bit.bio does not recommend using the complete medium for more than 4 days after preparation while stored at 4°C.

- **comp:GN+D: doxycycline supplemented** complete glutamatergic neuron medium

Reagent/ Media	For 10mL	For 50mL
comp: GN	10mL	50mL
Doxycycline (final conc. 1µg/mL)	5µL	25µL

Note: The supplemented complete medium is better prepared fresh before each feeding. bit.bio does not recommend using the supplemented complete medium for more than 4 days after preparation while stored at 4°C.

- **comp:GN+D+DAPT: doxycycline and DAPT supplemented** complete glutamatergic neuron medium

Reagent/ Media	For 10mL	For 50mL
comp: GN	10mL	50mL
Doxycycline (final conc. 1µg/mL)	5µL	25µL
DAPT (final conc. 10µM)	5µL	25µL

Note: The supplemented complete medium is better prepared fresh before each feeding. bit.bio does not recommend using the supplemented complete medium for more than 4 days after preparation while stored at 4°C.

bit.bio
The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
www.bit.bio

Customer support:
technical@bit.bio
orders@bit.bio

5.5 – Preparation of the PDL-Geltrex coating solution and coated vessels

To make PDL-Geltrex coated plates, treat culture vessels first with PDL solution and subsequently with Geltrex according to the protocol below.

Preparation of PDL coating solution:

Note:

- to avoid freeze-thaw cycles, aliquot the coating solution as appropriate for future use.
- the following coating solution is prepared using the recommended reagents in Appendix 5.2

1. Make up 50mL of 1x borate buffer, by diluting 2.5mL of the 20x stock with 47.5mL of sterile water.
2. Resuspend a 5mg vial of PDL in 50mL of the 1x borate buffer, for a working concentration of 100µg/mL.
3. PDL coating solution can be kept at -20°C for long-term storage.

PDL coating:

1. Calculate the total surface area to be coated.
2. Coat the surface area of your culture vessel with the PDL coating solution. We recommend the coating volumes shown in the table below (circa 100µL per cm^2).

Coating solution	384well	96well	24well	12well	6well	10cm dish
PDL	15µL	50µL	400µL	500µL	1mL	6mL

3. Incubate the coated plates, overnight at 37°C or for at least 3 hours at 37°C.
4. Aspirate PDL solution and then wash 3 times with sterile water. For each wash, use the same volume used for coating.
5. Aspirate the water and allow coated surfaces to dry completely in a laminar flow hood (without lids). This typically requires 30 – 60 mins.
6. Proceed with the Geltrex coating as described below.

For the preparation of Geltrex aliquots and PDL-Geltrex coated plates, please follow the manufacturer's instructions. In brief:

Preparation of Geltrex aliquots:

1. Remove Geltrex stock from -80°C and thaw on ice in a 4°C fridge overnight.
2. The next day, prepare aliquots according to foreseen use. In order to minimise further freeze thawing; store at -80°C.
   ⚠ Note: Geltrex solidifies quickly at temperatures above 4°C – keep Geltrex on ice at all times.
3. Depending on the volume, the smaller aliquots should take about 30 minutes to thaw while kept on ice.

Geltrex coating:

1. Calculate the total surface area to be coated.
2. Dilute the Geltrex 1:100 in chilled DMEM/F-12 (e.g. 100µL in 10mL).
3. Coat the surface area of your culture vessel with the Geltrex:DMEM/F-12 coating solution. We recommend the follow coating volumes (circa 100µL per cm^2):

Coating solution	384well	96well	24well	12well	6well	10cm dish
Geltrex:DMEM/F-12	15µL	50µL	400µL	500µL	1mL	6mL

4. Incubate the coated plates at 37°C for a minimum of 60 minutes.
5. Carefully aspirate off the excess Geltrex, then immediately plate the cells.

   Note: For long-term storage of Geltrex-coated plates, maintain the excess Geltrex in each well, and seal the plate with parafilm. Store at 4°C and use within 1 month.

bit.bio
The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
www.bit.bio

Customer support:
technical@bit.bio
orders@bit.bio

bit.bio
The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
www.bit.bio

Customer support:
technical@bit.bio
orders@bit.bio

Restrictions and liabilities

This document is provided "as is." Bit Bio Limited assumes no responsibility for any typographical, technical, or other inaccuracies in this document. Bit Bio reserves the right to periodically change information that is contained in this document; however, Bit Bio makes no commitment to provide any such changes, updates, enhancements, or other additions to this document to you in a timely manner or at all.

OTHER THAN THE EXPRESS LIMITED WARRANTY SET FORTH IN THIS USER'S GUIDE AND LIMITED USE LICENSES, BIT.BIO MAKES NO REPRESENTATIONS, WARRANTIES, CONDITIONS OR COVENANTS, EITHER EXPRESS OR IMPLIED (INCLUDING WITHOUT LIMITATION, ANY EXPRESS OR IMPLIED WARRANTIES OR CONDITIONS OF FITNESS FOR A PARTICULAR PURPOSE, NON-INFRINGEMENT, MERCHANTABILITY, DURABILITY, TITLE, OR RELATED TO THE PERFORMANCE OR NON-PERFORMANCE OF ANY PRODUCT REFERENCED HEREIN OR PERFORMANCE OF ANY SERVICES REFERENCED HEREIN.

This document might contain references to third-party sources of information, hardware or software, products or services and/or third-party websites (collectively the "Third-Party Information"). Bit Bio does not control, and is not responsible for, any Third-Party Information, including, without limitation the content, accuracy, copyright compliance, compatibility, performance, trustworthiness, legality, decency, links, or any other aspect of Third-Party Information. The inclusion of Third-Party Information in this document does not imply endorsement by Bit Bio of the Third-Party Information or the third party in any way. Bit Bio does not in any way guarantee or represent that you will obtain satisfactory results from using Bit Bio glutamatergic neurons as described herein. The only warranties provided to you are included in the Limited Warranty enclosed with this guide. You assume all risk in connection with your use of Bit Bio glutamatergic neurons.

Conditions of use

Your use of this product is subject to compliance with any applicable licensing requirements as detailed on our website at http://www.bit.bio, on the label or other documentation accompanying the goods. It is your responsibility to review, understand and adhere to any restrictions imposed by such statements.

Origin

bit.bio glutamatergic neurons are manufactured in the United Kingdom.

Copyright Notice © 2022 Bit Bio Limited. All rights reserved. This document may not be reproduced, distributed, modified or publicly displayed without the express written permission of Bit Bio Limited.

Limited Use License, Statement of Use, Terms of Sale

This product is provided by Bit Bio Ltd. under licensing rights from several entities (the "Licensors") including Cambridge Enterprise Limited, iPS Academia Japan, ID Pharma Co., TET Systems GmbH, ERS Genomics Limited, and Sigma-Aldrich Co. LLC. To the best of our knowledge, no additional licenses are required by the User other than the Single Use TET license. Use is limited as specified in the individual Limited Use License Notices or other license grant restrictions from each of the Licensors. All applicable license grant restrictions and terms are set forth in the Limited Use License supplied with each purchase of Product and available for download along with bit.bio's Statement of Use and Terms of Sale.

- Limited Use License: https://bit.bio/limited-use-license.pdf
- Statement of Use: https://bit.bio/statement-of-use.pdf
- Terms of Sale: https://bit.bio/terms-of-sale.pdf

Related products

ioGlutamatergic Neurons cat number: io1001
https://www.bit.bio/glutamatergic-neurons

bit.bio
The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
www.bit.bio

Customer support:
technical@bit.bio
orders@bit.bio