

# ioGlutamatergic Neurons

## User Manual

This user manual is applicable for the following products:

ioWild Type Cells™	
abcam Cat. No.	Product Name
ab303447	ioGlutamatergic Neurons*

ioDisease Model Cells™	
abcam Cat. No.	Product Name
ab303448	ioGlutamatergic Neurons HTT 50CAG/WT
ab307781	ioGlutamatergic Neurons TDP-43 M337V/M337V
ab307782	ioGlutamatergic Neurons TDP-43 M337V/WT
ab307783	ioGlutamatergic Neurons MAPT P301S/P301S
ab307784	ioGlutamatergic Neurons MAPT P301S/WT

\* ioGlutamatergic Neurons (ab303447), is the genetically matched (isogenic) control for the listed ioDisease Model Cells.

## Before starting

- Transfer the vials of ioGlutamatergic Neurons to liquid nitrogen or to -150°C immediately after receipt.
- bit.bio recommends reading this protocol in its entirety before the revival of the ioGlutamatergic Neurons.
- Before the revival of ioGlutamatergic Neurons, prepare the tissue culture plates or flasks coated with PDL-Geltrex (Appendix 6.5).
- The recommended reagents for the revival and maintenance of ioGlutamatergic Neurons can be found in Appendix 6.3. Ensure there is enough of each reagent for the full experiment before beginning.



If you have a question or need assistance, please refer to the product FAQs and resources pages, or email [technical@bit.bio](mailto:technical@bit.bio)

### Important information:

- ioGlutamatergic Neurons and the ioDisease Model Cells listed on page 1, referred to as ioGlutamatergic Neurons throughout this user manual, are for research 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 are cryopreserved in a medium containing DMSO: the safety data sheet (SDS) of dimethyl sulfoxide (DMSO) is available on request.
- ioGlutamatergic Neurons should only be used by personnel qualified in handling human biological materials following local health and safety regulations.

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## 1. Overview of ioGlutamatergic Neurons

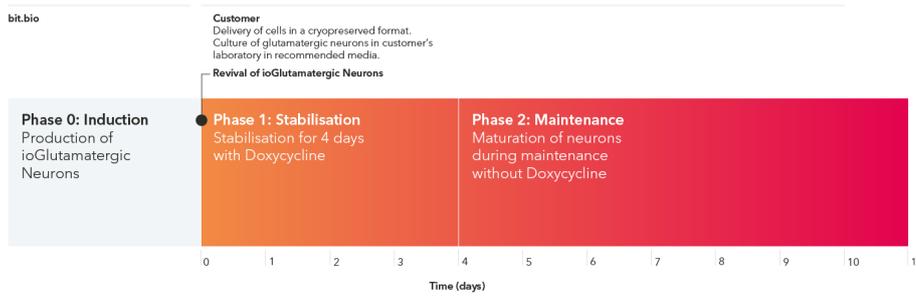
ioGlutamatergic Neurons mature rapidly into functional glutamatergic neurons after revival in the recommended medium. They are delivered in a convenient cryopreserved format and provide a homogeneous and reproducible model for human excitatory neurons.

The wild type ioGlutamatergic Neurons (ab303447) cultures consist mainly of glutamatergic neurons (>80%) characterised by the expression of the glutamate transporter genes VGLUT1 and VGLUT2.

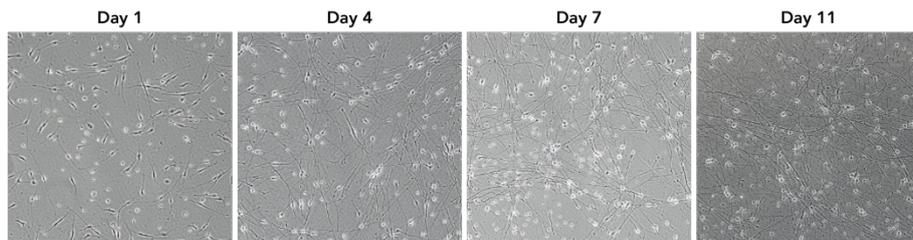
The minor remaining fraction of the neuronal population express marker genes of cholinergic neurons.

A bulk RNA-sequencing analysis of ioGlutamatergic Neurons (ab303447) shows that they have a rostral CNS identity and express the classical cortical marker genes FOXP1 and TBR1.

The protocol for the generation of ioGlutamatergic Neurons is a three-phase process (Table 1). Phase 0 - Induction is carried out at bit.bio before distribution (Figure 1A).



**Figure 1A**  
Schematic representation of the three-phase protocol to produce and culture ioGlutamatergic Neurons.



**Figure 1B**  
Brightfield images of ioGlutamatergic Neurons after revival over the course of the first 11 days of culture (day 1 to 11 post-thawing; 100X magnification). Images are representative of all products on page 1.

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<b>bit.bio</b>	<p><b>Phase 0 – Induction (Day -3 to 0):</b></p> <p>Human induced pluripotent stem cells (iPSCs) are exposed to an induction protocol. This leads to irreversible loss of pluripotency, and the synchronised homogeneous production of glutamatergic neurons. The ioGlutamatergic Neurons are subsequently cryopreserved for distribution.</p>
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<b>User</b>	<p><b>Phase 1 – Stabilisation (Day 0 to 4):</b></p> <p>The ioGlutamatergic Neurons are revived at the user’s laboratory using the recommended medium supplemented with doxycycline (96h) and DAPT (day 2-4) for sustained induction.</p> <p><b>Phase 2 – Maintenance (Day 4 onwards):</b></p> <p>Depending on assay requirements, the ioGlutamatergic Neurons can be used over different lengths of time in the maintenance medium.</p> <p>Note: ioGlutamatergic Neurons (ab303447) have been maintained up to 28 days in the above conditions without impairment to neuronal health, function, and culture attachment.</p>

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**Table 1**  
Description of the three-phase protocol for the production and culture of ioGlutamatergic Neurons.

## 2. QuickStart Guide

Read and perform the complete protocol (Section 3) at least once before using this guide.

<b>Before you begin</b>	<p>Prepare the cell culture surfaces. Appendix 6.5.</p> <hr/> <p>Prepare and warm the culture media. Appendix 6.4.</p>
<b>2.1</b> Cell thawing	<ol style="list-style-type: none"><li>1. Thaw the cryovial at 37°C (~2 min).</li><li>2. Transfer the cells to an empty 50mL tube.</li><li>3. Wash the cryovial with 1mL of <b>b:GN</b> and add it to the tube in a dropwise manner, agitating the tube occasionally.</li><li>4. Add a further 4mL of <b>b:GN</b>.</li><li>5. Centrifuge the cells at 200 x g for 3 min at room temperature.</li><li>6. Return cells to the biological safety cabinet and remove the supernatant by aspiration.</li><li>7. Add 1mL of <b>comp:GN+D</b> and gently resuspend the cells.</li></ol>
<b>2.2</b> Cell seeding	<ol style="list-style-type: none"><li>1. Count the cells including a cell viability marker.</li><li>2. Dilute the cell suspension to the required cell concentration using <b>comp:GN+D</b>.</li><li>3. Aspirate the Geltrex coating solution from the culture vessel(s).</li><li>4. Add the cell suspension to the culture vessel(s).</li><li>5. Transfer the culture vessel(s) to an incubator.</li><li>6. Gently cross-shake the plate once on the incubator shelf.</li></ol>
<b>2.3</b> Cell stabilisation & maintenance	<ol style="list-style-type: none"><li>1. Day 2: 48h post thawing, perform a 90% media change with fresh, pre-warmed <b>comp:GN+D+DAPT</b>.</li><li>2. Day 4: 96h post thawing, perform a 90% media change with fresh, pre-warmed <b>comp:GN</b>.</li><li>3. Day 6 onwards: perform a half-medium change with fresh, pre-warmed <b>comp:GN</b> every 48h.</li></ol>

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## 3. Culture of ioGlutamatergic Neurons

### Before starting:

- Each ioGlutamatergic Neurons vial contains either  $\geq 1.0 \times 10^6$  (small vial) or  $\geq 5.0 \times 10^6$  (large vial) viable cells. bit.bio recommends a minimum seeding density of 30,000 cells/cm<sup>2</sup>.
- Prepare enough tissue culture vessels with PDL-Geltrex coating prior to reviving the cryovial (Appendix 6.5).
- Warm-up the water bath to 37°C.
- Allow **basal glutamatergic neuron (b:GN) medium** to reach room temperature prior to thawing the cells (Appendix 6.4).
- Prepare the **complete glutamatergic neuron (comp:GN) medium** supplemented by **1 µg/mL doxycycline (D) (comp:GN+D)** for revival (Appendix 6.4).
- Prepare the cell viability marker and slides for counting the cells (step 3.2.1) before beginning. This will ensure there is minimal time for the cells to settle before counting.
- Remove the vials from liquid nitrogen immediately before step 3.1.1 and keep at -80°C or in dry ice. Do not store the cells at -80°C or in dry ice long term.

### Revival and culture procedures:

#### 3.1 – Cell thawing

1. Remove the cryovial from dry ice and immediately immerse into a 37°C water bath (or similar) while maintaining a constant gentle agitation. Avoid submerging the cap. Use of a floating microcentrifuge tube rack is recommended.



Minimise the number of vials being thawed at a time, to reduce time in suspension. Pool the cells in step 3.1.4 if thawing more than one vial of the same cell type.

2. Remove the cryovial 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 with 70% ethanol and take it to a biological safety cabinet.
4. Transfer the cells from each vial into an empty 50mL tube.
5. Carefully wash the cryovial with 1mL of **b:GN medium** and add the media to the tube in a dropwise manner, agitating the tube occasionally.



Freezing medium contains DMSO: minimise the time between thawing and centrifugation.

6. Add a further 4mL of **b:GN medium** in a dropwise manner, agitating the tube occasionally.
7. Centrifuge the cells at 200 x g for 3 minutes at room temperature.

8. Return cells to biological safety cabinet and carefully remove the supernatant by aspiration, taking care not to disturb the cell pellet.
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.

### 3.2 – Cell seeding (Day 0)

1. Count the cells including a cell viability marker. The typical recovery from one cryovial is  $\geq 1.0 \times 10^6$  viable cells (small vial) or  $\geq 5.0 \times 10^6$  viable cells (large vial).

Note: Perform 3 separate counts to ensure the average count is accurate.

2. 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. A seeding density of 30,000 cells/cm<sup>2</sup> is routinely used at bit.bio.

Plate format	Surface (cm <sup>2</sup> )	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

Note: For a protocol specific to 96 and 384 well plates please go to Section 4.



Well surface areas vary from supplier to supplier and should be adjusted accordingly.

3. Aspirate the Geltrex coating solution from the culture vessel(s).



Do not allow the Geltrex coating to dry out while seeding the plates. If necessary, aspirate and seed in batches.

4. Directly add the required volume of cell suspension to the culture vessel(s).



Gently mix the cell suspension periodically to ensure even cell distribution.

5. Immediately transfer the culture vessel(s) to a standard normoxic tissue culture humidified incubator at 37°C, 5% CO<sub>2</sub>.
6. 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).

### 3.3 – Cell stabilisation (Day 0 to 4) and maintenance (Day 4 onwards)

1. Day 2: 48h post thawing, gently aspirate 90% of the media and replace it with fresh pre-warmed **comp:GN+D medium supplemented with 10µM of DAPT (comp:GN+D+DAPT)**.



Carry out culture with special care as neuronal cells are prone to mechanical stress which may cause detachment.



Perform medium aspiration and addition slowly and on the side of the well, using micropipettes instead of serological pipettes.

2. Day 4: 96h post thawing gently aspirate 90% of the media and replace it with fresh pre-warmed **comp:GN medium** (no doxycycline).
3. Day 6 onwards: for optimal glutamatergic neuron maintenance, bit.bio recommends a half-medium change every 48h, i.e., replacing 50% of the medium with fresh pre-warmed **comp:GN medium** (no doxycycline).

## 4. Culture of ioGlutamatergic Neurons in 96 or 384 well plates

The following protocol has been optimised for the revival and culture of ioGlutamatergic Neurons 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 6.5).
2. Thaw the cells as per the standard protocol described in Section 3.1.
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/cm <sup>2</sup> )	384well (0.056cm <sup>2</sup> , 30µL)		96well (0.32cm <sup>2</sup> , 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<sup>2</sup> 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.



Do not allow the Geltrex coating to dry out while seeding the plates. If necessary, aspirate and seed in batches.

5. Pour the adjusted seeding cell suspension into a reservoir suitable for multichannel pipettes.

- Using a multichannel pipette, add 30 $\mu$ L or 100 $\mu$ L of the cell suspension into the wells of the 384 or 96 well plate, respectively.



Gently mix the cell suspension periodically to ensure even cell distribution.

- Transfer the culture plate into the standard normoxic tissue culture humidified incubator at 37°C, 5% CO<sub>2</sub>.
- Day 2: 48h post thawing, gently aspirate 90% of the media and replace it with fresh pre-warmed **comp:GN+D+DAPT medium**.



Carry out culture with special care as neuronal cells are prone to mechanical stress which may cause detachment.



Perform medium aspiration and addition slowly and on the side of the well, using micropipettes instead of serological pipettes.

- Day 4: 96h post thawing, gently aspirate 90% of the media and replace it with double the original volume of fresh pre-warmed **comp:GN medium** (no doxycycline). (200 $\mu$ L and 60 $\mu$ L into the wells of a 96 and 384 well plate, respectively).
- Day 6 onwards: for optimal glutamatergic neuron maintenance, bit.bio recommends a half-medium change every 48h, i.e., replacing 50% of the medium with fresh **comp:GN medium** (no doxycycline).

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## 5. Co-culture of ioGlutamatergic Neurons with Astrocytes

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 (Appendix 6.5). bit.bio recommends a 1:1 co-culture ratio of ioGlutamatergic Neurons and astrocytes, and a seeding density of 30,000 cells/cm<sup>2</sup> of each cell type (total of 60,000 cells/cm<sup>2</sup>).



If these cells are being used for a functional experiment, e.g. MEA, the ideal seeding density may be different. Refer to the assay manufacturer's guidelines for their recommendations.

- Warm-up the water bath to 37°C.
- Allow **basal glutamatergic neuron (b:GN) medium** to reach room temperature prior to thawing the cells (Appendix 6.4).
- Prepare the **complete glutamatergic neuron (comp:GN) medium** supplemented by **1 µg/mL doxycycline (comp:GN+D)** for revival on Day 0. Do not make this media in advance (Appendix 6.4).
- Prepare astrocytes for seeding according to manufacturer/author's protocol, in parallel to step 5.1.2 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<sub>2</sub>, occasionally shaking the cell suspension. Make sure the cap of the vial or tube is not fully closed. Proceed immediately to step 5.1.3.

### Revival and culture procedures:

#### 5.1 – Cell thawing and seeding (Day 0)

1. Pre-coat the required wells with PDL-Geltrex (Appendix 6.5).
2. Thaw the cells as per the standard protocol described in Section 3.1.
3. Resuspend astrocytes and ioGlutamatergic Neurons 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<sup>2</sup> of each cell type is routinely used at bit.bio.
4. Gently mix both cell suspensions to achieve a homogeneous 1:1 ratio mixed-cell suspension.
5. Aspirate the Geltrex coating solution from the culture vessel(s).



Do not allow the Geltrex coating to dry out while seeding the plates. If necessary, aspirate and seed in batches.

6. Directly add the required volume of cell suspension to the culture vessel(s).



Gently mix the cell suspension periodically to ensure even cell distribution.

7. Immediately transfer the culture vessel(s) to a standard normoxic tissue culture humidified incubator at 37°C, 5% CO<sub>2</sub>.
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).

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

1. Day 2: 48h post thawing, gently aspirate 90% of the media and replace it with fresh pre-warmed **comp:GN+D+DAPT medium**.



Carry out culture with special care as neuronal cells are prone to mechanical stress which may cause detachment.



Perform medium aspiration and addition slowly and on the side of the well, using micropipettes instead of serological pipettes.

2. Day 4: 96h post thawing, gently aspirate 90% of the media and replace it with fresh pre-warmed **comp:GN medium** (no doxycycline). A single addition of 2µM Cytarabine (ara-C) to arrest further astrocyte growth is recommended at this stage.
3. Day 6 onwards: for optimal astrocyte-glutamatergic neuron co-culture maintenance, bit.bio recommends a half-medium change every 48h, i.e., replacing 50% of the medium with fresh pre-warmed **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.

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## 6. Appendices

### 6.1 – Equipment required

- Biological safety cabinet
- Normoxic cell culture incubator (37°C, 5% CO<sub>2</sub>)
- 37°C water bath
- Haemocytometer or calibrated automatic cell counter
- Liquid nitrogen storage unit
- Standard tissue culture wares (pipettes, tips, culture plates)
- Bench top centrifuge

### 6.2 – 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 (50X)	ThermoFisher	17504044	-20°C to -80°C
Glutamax	ThermoFisher	35050061	2°C to 8°C
2-Mercaptoethanol (50mM)	ThermoFisher	31350010	2°C to 8°C
NT3	Biotechne R&D Systems	267-N3-025	-20°C to -80°C
BDNF	Biotechne R&D Systems	248-BDB-005	-20°C to -80°C
DAPT	Biotechne R&D Systems	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

\*Filter the reconstituted Bovine Serum Albumin prior to use.

### 6.3 – 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 manufacturer's protocol	10µM 0.5µL of stock solution per 1mL of medium
Doxycycline	2mg/mL (2000X solution): To prepare, reconstitute 20mg in 10mL of sterile water	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 sterile water	2µM 0.1µL of stock solution per 1mL of medium

## 6.4 – Preparation of glutamatergic neuron medium

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

Reagent/Media	For 200mL	For 500mL
Neurobasal	197.9mL	494.75mL
Glutamax (100X)	2mL	5mL
2-Mercaptoethanol (final conc. 25µM)	100µL	250µL

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

Note: A 500mL bottle of medium rarely contains exactly 500mL. Please decant the precise volume of media before adding the supplements to guarantee the correct concentration.



Aliquot the basal medium at 4°C prior to use, so the entire bottle is not being warmed and cooled repeatedly.

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

Reagent/Media	For 50mL	For 200mL
b:GN	49mL	195.9mL
B27 (50X)	1mL	4mL
NT3 (final conc. 10ng/mL)	10µL	40µL
BDNF (final conc. 5ng/mL)	25µL	100µL



Do not use the complete medium for more than 4 days after preparation, and aliquot it at 4°C prior to use, so the entire bottle is not being warmed and cooled repeatedly.

- **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



Add fresh supplements to a new aliquot of the complete medium on each day it is required.

- **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



Add fresh supplements to a new aliquot of the complete medium on each day it is required.

## 6.5 – 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 6.2
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.



Vigorously mix the PDL and borate buffer.

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<sup>2</sup>).

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

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 minutes.
6. Proceed with the Geltrex coating as described below.



Ensure the PDL is completely dry before moving to the next step.

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. Store at -80°C in order to minimise further freeze thawing.



Geltrex polymerises quickly at temperatures above 4°C - always keep Geltrex below 4°C.

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 following coating volumes (circa 100µL per cm<sup>2</sup>):

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

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.