Axiogenesis growing portfolio on human iPSC-derived cells
Overview

hiPSC (from Axiogenesis/from clients/CRISPR/…)

- Cor.4U
- CorV.4U
- Fibro-Cor.4U
- Cardiac-Tissue.4U
- Fibroblasts WT
- Fibroblasts MT
- NPC
- Dopa.4U
- Peri.4U
- CNS.4U
- Astro.4U
Overview (Current Products)

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- Fibro-Cor.4U
Current „Neural-Products“

Dopa.4U - Dopaminergic Neurons

Peri.4U - Peripheral Neurons
### Dopa.4U - Dopaminergic Neurons

| **Beta-3-tubulin**  
| (neuron-specific microtubuli) | **Tyrosine Hydroxylase**  
| (dopaminergic neuron-specific protein) |

![Image of Beta-3-tubulin](image1.png)  
![Image of Tyrosine Hydroxylase](image2.png)
Dopa.4U - Dopaminergic Neurons

**MAP-2**
(neuron-specific microtubule-associated protein 2)

**Synaptophysin**
(presynaptic)
Dopa.4U - Patch Clamp - Current clamp mode (whole cell configuration)

Dopa.4U cells exhibit spontaneous action potentials

Spontaneous action potentials in current clamp mode (resting potential of an inactive cell is around -80 mV)

Spontaneous action potential (higher magnification)
Dopa.4U - Patch Clamp - Voltage clamp mode (whole cell configuration)

Overlay of current traces showing the responses to voltage pulses from $-60 \text{ mV}$ to $+60 \text{ mV}$ in $5 \text{ mV}$ increments.

Dopa.4U exhibit Sodium Currents ($0.5 \text{ nA} - 2 \text{ nA}$)
Dopa.4U - Patch Clamp - Measurement of single channels
Dopa.4U were seeded (30,000 cells/cm²) onto cover slips and measured after 11 to 49 days in culture.

Dopa.4U cells maintain sodium currents during long-term culture
Dopa.4U - MEA Recordings

**MEA recording station Axion Biosystems**

**Maestro System, 12 well MEA**
Dopa.4U - MEA Recordings

12 Wells – Heatmap (spontaneous activity)

Activity at 7 days in vitro

Bursting neurons

activity of one well (7div)
Dopa.4U - Comparison of the Spontaneous Activity Patterns:

Primary Mouse Frontal Cortex – 28 days in vitro

Primary Mouse Midbrain – 28 days in vitro

Primary Mouse Hypothalamus – 28 days in vitro

Dopa.4U human iPSC-derived dopaminergic neurons

7 days in vitro

14 days in vitro

60 sec
Peri.4U - Peripheral Neurons

**Beta-3-tubulin**
(neuron-specific microtubuli)

**MAP-2**
(neuron-specific microtubule-associated protein 2)
Peri.4U - Peripheral Neurons

**MAP-2**
(neuron-specific microtubule-associated protein 2)

**Peripherin**
(peripheral neuron-specific intermediate filament)
Peri.4U - Neurite Outgrowth

**PC12**
- 100 pM Vinblastine
- 1 µM Vinblastine

**Vinblastine**
- Dose-Response Curve
- Log[Dose], µM
- Rel. Neurite Length

**Peri.4U**
- 100 pM Vinblastine
- 100 nM Vinblastine

**Note:**
- The graphs show the effect of different concentrations of Vinblastine on neurite outgrowth in PC12 and Peri.4U cells. The data points indicate the relative neurite length at various dose levels, with statistical values (e.g., 0.01297 and 0.003541) likely representing significance levels or error margins.
Peri.4U - Peripheral Neurons

MAP-2 (neuron-specific microtubule-associated protein 2)

Synaptophysin (presynaptic)
Peri.4U - Peripheral Neurons

MAP-2
(neuron-specific microtubule-associated protein 2)

PSD95
(postsynaptic)
Peri.4U - Patch Clamp (whole cell configuration)
Peri.4U - MicroElectrode Array (MEA)

Peri.4U - 3 days *in vitro*

Anthony Nicolini, Axion Biosystems
Peri.4U - MEA Tox

Characterization

Product/Format

Service

Company

Peri.4U - hiPS neurons from Axiogenesis

Cultivation

• Coating with PEI and laminin
• MEAs with PEI and the Peri.4U neurons with laminin
• 2 µl cell suspension per well (density 5000 cells/µl) ⇒ 10000 cells per well ⇒ 500000 cells/cm²

• Recordings were performed at DIV 12-20
• Similar protocol used for seeding neurons on cover slips for manual patch clamp recordings

Effect of DMSO (Vehicle control)

Effect of fipronil

1E-7 1E-6 1E-5 1E-4

0

20

40

60

80

100

120

140

DMSO

Fipronil

Norm. mean spike frequency in burst [%]

Concentration of fipronil

IC₅₀ = 4.4 ± 1.6 µM

DMSO

Fipronil

Norm. mean burst duration [%]

Bursts per min.

Burst duration

Spike frequency in burst

n=24 recordings

n=5

Fipronil (1µM)

Fipronil (10µM)

30 sec

α

I Na amplitude (% control) 95 ± 5.2% (9) 63.3 ± 12.9% (3) <0.05

Resting membrane potential 1.5 ± 0.5 mV (13) 1.8 ± 1.7 mV (3) >0.05

Firing properties no effect (13) reduction to 1 AP (3)

Rat cortical neurons

Decreased Neuronal Activity with Fipronil:

- Rat cortical neurons ≥ 10µM
- Other Neuron Supplier ≥ 50µM
- Peri.4U IC₅₀=4.4 ± 1.6µM

Manual patch clamp 0.1% DMSO (n) 30 µM Fipronil (n)
- **Histamine** (ligand of H3 Receptor expressed in peripheral neurons)
- **Glutamate** (ligand for metabotropic and ionotrophic glutamate receptors as the NMDA receptor in peripheral neurons)
- **Serotonin** (ligand for 5-HT3 receptor, specific for peripheral neurons)
- **NMDA** (ligand for NMDA receptor expressed in peripheral neurons)
- **ATP** (Ligand for purinergic receptors P2RX4 and P2RX7 expressed in peripheral neurons)
- **PBS** (control)
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- Dopa.4U
- Peri.4U
- DopCA.4U
- PeriCA.4U

Fibroblasts WT

Fibroblasts MT

NPC
- CNS.4U
- Astro.4U
PeriCA.4U and DopaCA.4U - Integrated $\text{Ca}^{2+}$ Sensor

PeriCA.4U: 10 µM Ionomycin
Overview (ß-Product)

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- Cor.4U
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Fibro-Cor.4U

Cardiac-Tissue.4U

CorV.4U

- Dopa.4U
- Peri.4U
- DopaCA.4U
- PeriCA.4U

NPC

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Fibroblasts WT

Fibroblasts MT
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Company

Characterisation

Applications

Services

Formats
CNS.4U - human iPSC derived Central Nervous System Cells

TuJ1  S100β  Hoechst

Neurons / Astrocytes (50% / 50%)
- Glutamatergic Neurons (40-45%)
- GABAergic Neurons (35-40%)
- Dopaminergic Neurons (10-15%)
Astro.4U - human iPSC derived Astrocytes

<table>
<thead>
<tr>
<th>Protein</th>
<th>Description</th>
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<tbody>
<tr>
<td>GFAP</td>
<td>(Glial fibrillary acidic protein)</td>
</tr>
<tr>
<td>S100B</td>
<td>(S100 calcium-binding protein B)</td>
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</tbody>
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Overview (Services)

hiPSC (from Axiogenesis/from clients/CRISPR/…)

„On Demand“ Cell-Service

Banking & Scale Up
Gene Editing (CRISPR/CAS9)
Patient-derived
Co-development

NPC

Dopa.4U
Peri.4U
DopaCA.4U
PeriCA.4U
CNS.4U
Astro.4U

Fibroblasts WT
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