

HUMAN iPSC-DERIVED MIXED CNS CELLS

ADVANTAGES

- ▶ Highly physiological neural networks
- ▶ Long term synchronous network activity
- ▶ Suitable as seizure liability model

Evotec's human iPSC-derived mixed CNS cells are comprised of two key cell types of the human central nervous system (CNS): Neurons and astrocytes. The cells are simultaneously generated from neural precursor cells and recapitulate the interplay between excitatory and inhibitory neurons. Their rapid formation of neuronal networks, prolonged viability in culture, and availability in unlimited volumes, make Evotec's mixed CNS cells advantageous compared to currently used primary cells and animal models for neuroscience-related applications. Our cell mix represents an ideal tool for early and late drug discovery for neurodegenerative disease and epilepsy, *in vitro* neurotoxicity assays, and disease modeling, through electrophysiological, metabolic and imaging-based assays. Evotec's human iPSC-derived mixed CNS cells have been validated through the FDA HESI Neutox initiative for *in vitro* seizure liability assessment.

Highly physiological neural networks

Evotec's mixed CNS cells are comprised of neurons (glutamatergic, GABAergic and dopaminergic) and astrocytes that rapidly form neuronal networks.

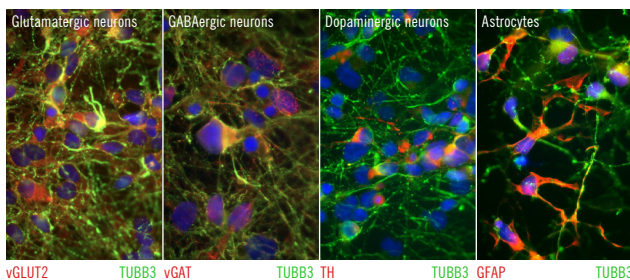


Figure A: Cell type composition. Evotec's mixed CNS cells form physiological networks comprised of dopaminergic (TH, red), glutamatergic (vGLUT2, red) and GABAergic (vGAT, red) neurons (TUBB3, green) as well as astrocytes (GFAP, red).

Long term synchronous network activity

Evotec's mixed CNS cells exhibit long-term synchronous network activity, which can be assessed by microelectrode array (MEA) technology.

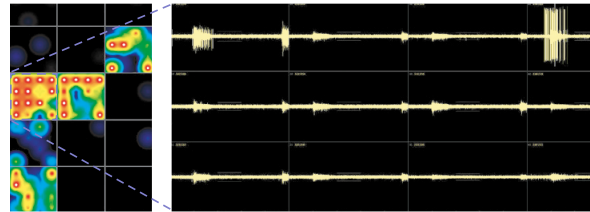


Figure B: Microelectrode array (MEA) technology, 34 days after thawing. Image shows heat map of activity (left). Inset of 1 well with 16 electrodes reveals highly synchronous network bursts (right).

Suitable as seizure liability model

Evotec's mixed CNS cells show dose dependent responsiveness to seizure-related compounds.

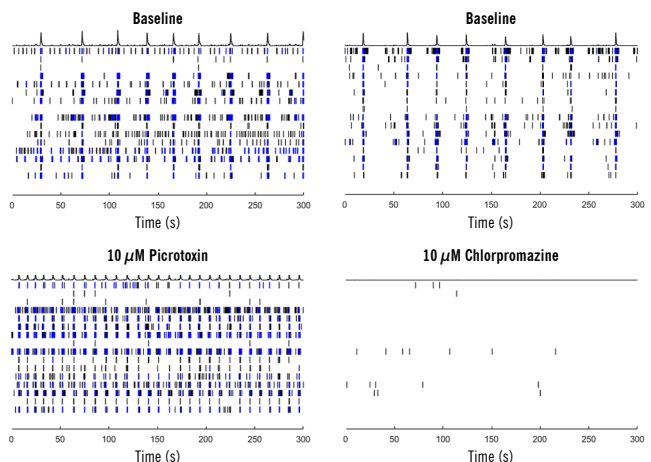


Figure C: Evotec's mixed CNS cells were treated with GABA receptor blocker Picrotoxin (left) or anti-psychotic Chlorpromazine (right). Picrotoxin increases the number of network bursts, while Chlorpromazine reduces the mean activity and network burst frequency in a reversible manner.

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PRODUCT SPECIFICATIONS

Cell type: Evotec's human iPSC-derived mixed CNS cells are a heterogeneous group of neuronal and glial cells (35–40% glutamatergic, ~30% GABAergic, 10–15% dopaminergic and 10–15% astrocytes)

Production technology: Scalable; large lot sizes with minimal batch-to-batch variability guaranteed by an extensive quality control of identity (immunostaining) and functionality (MEA analysis)

Vial size: 2 M cryopreserved cells; alternative sizes on request

Purity: Heterogeneous cultures

Morphology: Post-mitotic neural phenotype

Assay window: Neuronal spike activity (MEA after day 10, calcium oscillations (FDSS μ Cell) and network burst activity (MEA) at week 4–5

Electrophysiology: Robust spontaneous and burst-like neuronal activity of mixed CNS cells neurons present in MEA recordings. Neuronal network formation indicated by calcium oscillations and network burst activity established in cultivation week 4

Seizure liability assays: Dose-dependent responsiveness to seizure-related compounds, i.e., positive control picrotoxin increases number of network bursts, while negative control phenytoin reduces mean activity and network burst frequency

VALIDATED ASSAYS AND PROTOCOLS

Evotec's mixed CNS cells are integrated into a variety of validated assays on dedicated platforms that can be implemented in drug discovery and development for safety and efficacy evaluation of novel compounds:

- ▶ Microelectrode array (MEA) assays
- ▶ Immunocytochemistry
- ▶ High-throughput calcium transient assays
- ▶ Seizure liability testing
- ▶ Manual patch clamp assays
- ▶ High Content Imaging (HCI)
- ▶ Screening / profiling of small molecules, biologics, and antisense / siRNA screening

Evotec offers easy-to-follow protocols for most of these validated assays and their corresponding platforms. Reach out to enquiries-cologne@evotec.com for more information on our application notes and manuals.

EVOTEC *IN VITRO* iPSC RESEARCH SERVICES

Evotec further develops induced pluripotent stem cell (iPSC) based models and assays for efficacy and safety applications, in particular disease modeling and customized cell type development. We offer flexible entry and exit points for our clients and partners, who can choose only one or up to all of the following building blocks:

- ▶ Evotec or client iPSC lines
- ▶ Expansion & banking
- ▶ Genetic engineering
- ▶ Custom differentiation
- ▶ Disease modeling & exploratory research
- ▶ Compound screening & profiling

Do contact us to discuss how our iPSC technology can accelerate your drug development process.

