StemRNA™ Neuro

iPSC-derived Human Neurons

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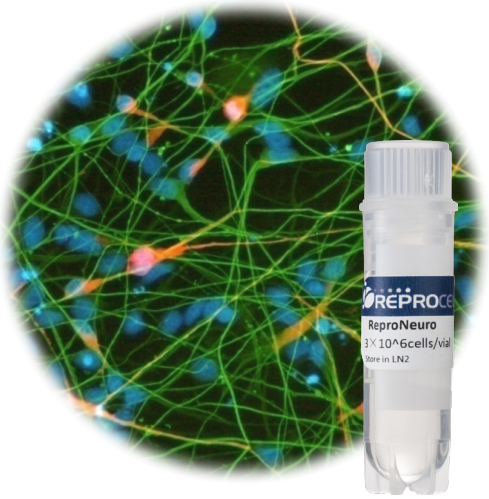


Stem Cell–derived Human Neurons

The availability of live human brain cells for research was made ethically possible by the advent of pluripotent stem cell technologies. Such cells are now used increasingly for drug toxicology, dementia-related disease modeling and brain physiology research. StemRNA Neuro* cells from REPROCELL are differentiated using proprietary technologies that result in a mixed population of neuronal cell types. Two alternative Alzheimer disease-models are also available, one patient-derived and the other an engineered mutation. Experimental results showing impairment of neurite outgrowth in the Alzheimer disease cells provides validation that StemRNA Neuro cells are a functional model and smart choice for your research.

StemRNA Neuro Human Neurocytes

- World's first commercially available iPSC-derived human neurons
- Easy to use and culture; enough cells provided for one full 96 well plate
- Reach phenotypic maturity after two weeks in culture
- Displays highly complex networked morphology with synaptic junctions
- Functional electrophysiology can be observed by MEA or patch-clamp
- Alzheimer disease patient-derived and engineered mutant versions are available
- Clonally derived thereby offering highly consistent performance and low lot-to-lot variation
- Stable phenotype and functionality up to several months in culture

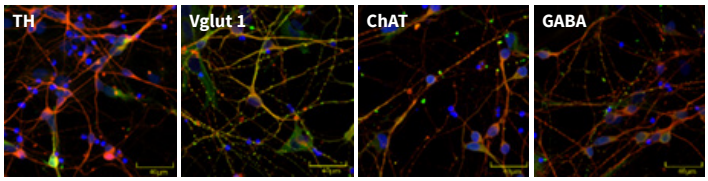
Product Overview

StemRNA Neuro is supplied as a frozen vial of late stage disrupted neurosphere progenitor cells in cryopreservation medium. Derived from a healthy 32 year old white male donor, the original iPS cells were reprogrammed by REPROCELL's advanced mRNA reprogramming technologies. One vial of StemRNA Neuro contains at least 3×10^6 viable cells, which is enough to seed a confluent 96-well culture plate. When plated and grown for more than two weeks in Neuro Culture Medium (RCDN101), the StemRNA Neuro cells form a network of mature neurons that develop increasingly dense synaptic connections over time.

* StemRNA Neuro was formerly known as ReproNeuro.

Culturing **StemRNA Neuro** Human Neurons

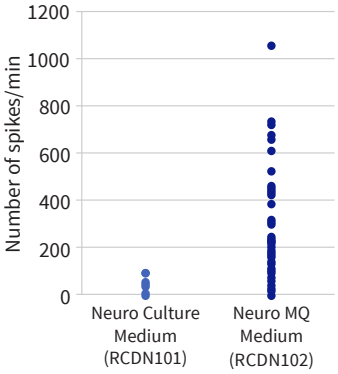
StemRNA Neuro is a single-cell suspension of late-stage progenitor cells prepared from disrupted neurospheres. When plated in 2D cell culture at recommended densities (about 1 × 10^5 cells/cm^2), the cell separation is optimal to promote neurite outgrowth and synaptic contact with adjacent cells. StemRNA Neuro cells can also be reassembled into neurospheres by plating in low-attachment U-bottom plates or similar spheroid-forming culture vessels. Cultivation of cells or re-associated neurospheres on scaffolds such as Alvetex™ can induce 3D structural networks. It is recommended to use Neuro Culture Medium or Neuro MQ Medium to culture StemRNA Neuro human neuronal cells.



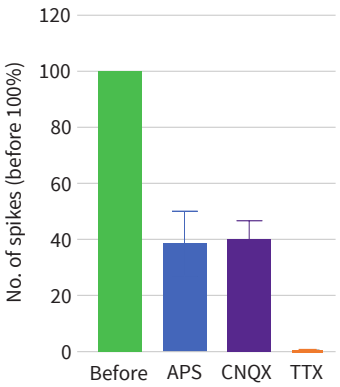
StemRNA Neuro cells in 2D culture. Cells were thawed and plated for 14 days in Neuro Culture Medium on plates that were pre-incubated with Neuro Coat. All panels were stained with a fluorescent βIII-tubulin antibody ad one other neuro-subtype specific antibody. TH = anti-tyrosine hydrolase specific for dopaminergic neurons; ChAT = anti-choline acetyltransferase specific for Cholinergic neuron; Vglut1 = vesicular glutamine trans-porter 1 specific for Glutamatergic neurons; GABA = anti-GABA specific for GABAergic neurons.

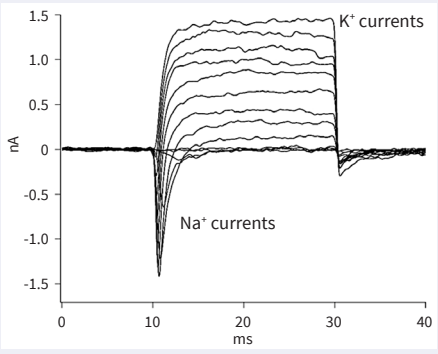
Experimental Analysis of **StemRNA Neuro** Cell Signaling

To analyze cell signaling and communication among StemRNA Neuro cells, various instruments can be used to measure electrical action potentials. These include multi-electrode array (MEA) systems, patch clamp systems and ion flux fluorescent measurement devices for observing intracellular calcium ion release.

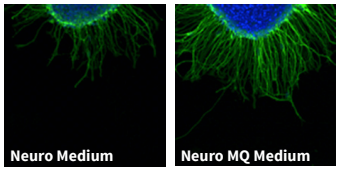


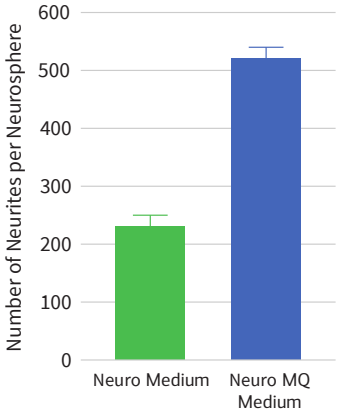
MEA Analysis is Enhanced by Neuro MQ Medium.
Action potentials for StemRNA Neuro cells are enhanced in frequency and intensity by maturation and growth in Neuro MQ Medium (*left*). The boosted activity allows for sensitive detection of drugs that down-regulate the spontaneous electrical potential correlating with published clinical data (*right*).





Auto Patch Clamp Validation of StemRNA Neuro.
StemRNA Neuro cells exhibit typical potassium outward and sodium inward ion currents. Data was collected on the Nan]i[on Patchliner™ instrument. A holding potential of –80 mV with step protocol at 10mV increments up to +40 mV is shown.





Neuro MQ Medium Enhances Neurite Outgrowth in 2D Cell Culture.
StemRNA Neuro cells were first reformed into neurospheres and then allowed to attach to plates previously treated with Neuro Coat. Both neurosphere formation and incubation were performed in either Neuro Culture Medium or Neuro MQ Medium. The cells were stained with DAPI (blue) and anti-TUJ-1 (green) fluorescent detection reagents. Images were acquired and neurites per neurosphere were determined using ImageJ software. Images and data analysis is shown (*left*).

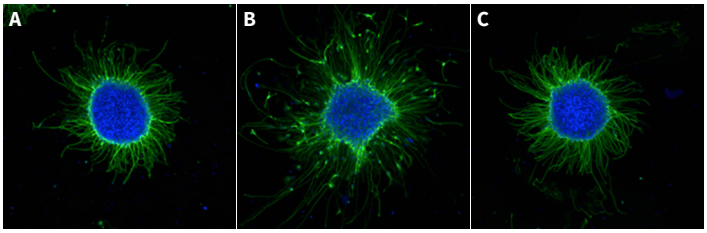
StemRNA Neuro Alzheimer Disease Mutants

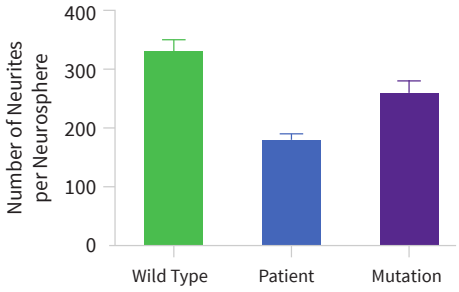
StemRNA Neuro AD-Mutation

Isogenic with the original StemRNA Neuro iPSC-strain, this cell is a transgene expression mutant carrying an engineered P117L mutation in the Presenilin 1 (PS1) gene. Consequently, it expresses an aberrant Aβ42 peptide fragment. This mutation is characteristic of the AD3 (Type 3) familial form of Alzheimer's disease.

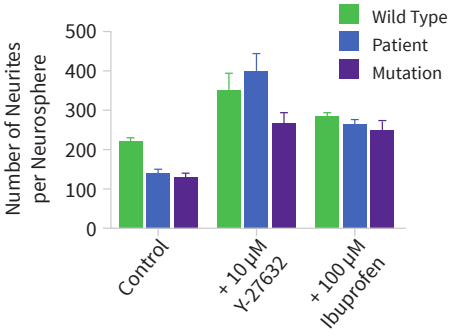
StemRNA Neuro AD-Patient

The StemRNA Neuro AD-Patient iPSC-strain was derived from an 94-year old male with Alzheimer's disease. The cells expresses a R62H mutation within the Presenilin 2 (PS2) gene. This mutation is characteristic of the AD4 (Type 4) familial form of Alzheimer's disease. It is a naturally occurring mutation.

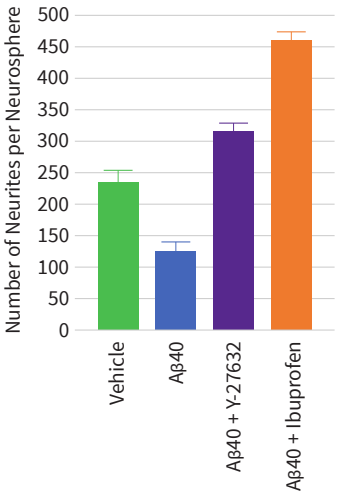




Neurite Outgrowth in 2D Culture. (**A.**) StemRNA Neuro, (**B.**) StemRNA Neuro AD-patient and (**C.**) StemRNA Neuro AD-mutant cells were first reformed into neurospheres and then allowed to attach to plates previously treated with ReproNeuro Coat. The cells were stained with DAPI (blue) and anti-TUJ-1 (green) fluorescent detection reagents (above). Image analysis with ImageJ software was used to quantitate neurites per neurosphere. Graphic representation of the results is shown at the right.

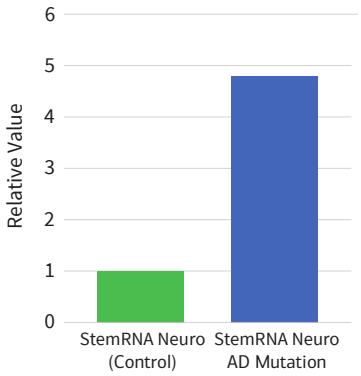


Inhibition of RhoA or ROCK Signaling can restore Neurite Outgrowth from Alzheimer Disease Phenotype Cells.
Activation of the Nogo receptor (NgR) pathway on nerve cells is known from the literature to decrease neuritogenesis and increase amyloid beta peptides levels. Amyloid beta peptides are also known to bind to NgR. RhoA and ROCK, and are part of the NgR transduction pathway that influences neurite formation. Inhibition of RhoA by ibuprofen and ROCK by Y-27632 has been shown to reduce amyloid beta petides levels and to protect nerve cells from amyloid-associated toxicity. StemRNA Neuro cell neurospheres, when attached to a 2D plate surface and treated with 10 µM Y-27632 and 100 µM ibuprofen, show enhance neurite formation as determined by image analysis using ImageJ software (data on left). This *in vitro* phenotypic response is consistent with published reports on natural and other iPSC-derived neuronal cell disease models.



Exogenous Amyloid β peptide (Aβ40) Suppresses Neurite formation.
Compared to the control with vehicle alone, StemRNA Neuro cells grown in the presence of exogenously added Aβ40 peptide demonstrate a reduction, by almost half, of the number of neurites formed by neurospheres when plated in 2D culture. The addition of 10 µM Y-27632 or 100 µM ibuprofen overcome the inhibitory effect to levels that exceed vehicle alone (left).

Amyloid β peptide (Aβ42/Aβ40) Secretion by StemRNA Neuro.
Compared to StemRNA Neuro (control), the StemRNA Neuro AD-Mutation cells express an elevated Aβ42/ Aβ40 peptide ratio into the cell culture medium as detemined with the AlphaLISA® Human Amyloid β Immuno-detection Kit (Perkin Elmer). There is a nearly 5-fold increase in the ratio, which is consistent with published literature concerning Alzheimer disease (right).



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Human Neuronal Cell Products and Services

Product Name	Quantity	Catalog Number
StemRNA Neuro Human iPSC-derived Neurons	1 vial (3×10^6 cells)	RCDN001N
StemRNA Neuro AD-mutation	1 vial (3×10^6 cells)	RCDN002N
StemRNA Neuro AD-patient	1 vial (3×10^6 cells)	RCDN003P
Neuro Culture Medium	40 mL	RCDN101
Neuro MQ Medium	40 mL	RCDN102
Neuro Coat	150 µL	RCDN201
Stemolecule™ Y-27632	2 mg 10 mg	04-0012 04-0012-10
Customized Cell Types – Human Neurons, etc.	Inquire	Custom service

StemRNA Neuro MQ Medium

StemRNA Neuro MQ Medium is a high performance culture medium designed for robust detection of spontaneous electrical action potentials of human neurons when analyzed by using Multi-Electrode Array (MEA) instrumentation. “MQ” means MEA-Qualified, referring to a critical *Quality Control* step for certification of the medium. A key component of StemRNA Neuro MQ Medium is the added supplement of rat astrocyte-conditioned medium. Consequently, the magnitude and the frequency of spontaneous electrical activity of the neurons in culture is significantly enhanced. This can be of benefit when investigating the *in vitro* modulation of electrical activity by drugs or other factors.

Custom Engineered Human Neurons

REPROCELL has technology experts who routinely make induced pluripotent stem cells (iPSC) lines and differentiated cell types. Using our latest footprint-free Stemgent StemRNA reprogramming technology, your custom iPSC line will be of the highest quality, stability and pluripotency. Whether you are interested in control strains, specific genetic backgrounds, dementia disease models or genome-edited cell lines, with our custom services you can leave the development to experts. We can even source the human tissue for you from our network of collection sites through BioServe resources.

Contact us to discuss research collaborations such as custom production of unique iPSC-derived cell types according to your specific needs.

Acknowledgement: The neurite outgrowth data shown in several figures of this brochure were kindly provided by the laboratory of Professor Stefan Przyborski and his postgraduate student Kirsty Clarke, Dept. of Biosciences, Durham University, Durham, UK.