NEURON BIO

Service Division

Develops research projects for public institutions and pharmaceutical, biotechnology, agro-food and animal health companies

Neuron Bio offers preclinical R&D services for the biomedicine, toxicological and agro-food sector, developing tailor-made projects through the use of *in vitro* and *in vivo* models (efficacy and safety, behavior studies …) in multiple study areas. We also have the capability to create transgenic animals for the study of any human disease.

The comprehensive offer of Neuron Bio is highly demanded by national and international companies that outsource part of their R&D projects. Our final goal is to assist the client in the evaluation of its products under development with the aim of a later successful market entry.

NEURON BIO

Screening Platform

Automated *in vitro* assays for a quick and efficient evaluation of functional and security properties of any compound

Your integrated solution for high-quality pre-clinical studies on the right path towards a regulatory agency approval. Neuron Bio offers standard services and tailor-made assays with regard to the specific strategy of each client and according to the guidelines determined by the regulatory agencies

In silico

Prediction of (a) Molecular drug-properties, (b) Bioactivity score for the most important drug targets and (c) Blood-Brain Barrier (BBB) permeability of a molecule.

In vitro

Mycoplasma Detection

Neuron Bio guarantees its customers an effective control of their laboratory samples by detecting almost 100% of the mycoplasma strains that produce these contaminations.

BBB Permeability

Analysis of Blood-Brain Barrier (BBB) permeability by PAMPA (Parallel Artificial Membrane Permeability Assay). PAMPA is an *in vitro* model of passive, transcellular permeation of BBB.

Antioxidant Capacity

Study of antioxidant capacity *in vitro* measured by ORAC (Oxygen Radical Absorbance Capacity), TEAC (Trolox Equivalent Antioxidant Capacity) and ABTS (Cation Radical Decolorization) assays.

Multiplex Assays

Multiplex Luminex® assays for quantitification and detection of cytokine and signal transduction molecules. These assays are designed to measure simultaneously multiple targets in each sample (up to 41), with superior performance and reproducible results than ELISA technique.

Cell Platform

Safety

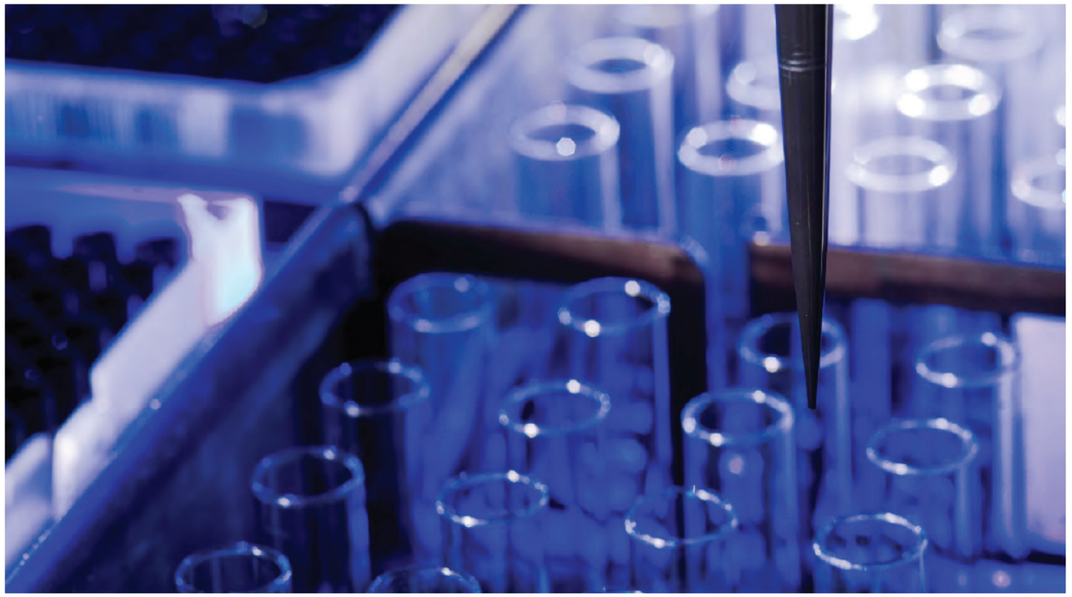
Study of citotoxicity in cell cultures of epithelial, hepatic and neuronal origins from different species (human, mouse, rat, canine…).

Protection from Cell Death

- Study of the protective capacity against cell death by oxidative stress, UPR-stress, cell cycle arrest and others by metabolic assays measuring (WST-1, LDH).
- Study of protective capacity against cell death by Real-Time Cell Analyzer (RTCA).
- Analysis of anti-apoptotic capacity by propidium iodide staining and flow cytometry. Apoptotic DNA fragmentation is measured in an apoptotic cell model.
- Analysis of anti-apoptotic capacity by caspase-3 activity measurement. Caspase-3 activity is measured by a fluorescent probe assay in an apoptotic cell model.

Protection from Inflammation

- Study of anti-inflammatory capacity measuring cytokines produced in human monocytes. Cell cultures are treated with inflammatory agents and cytokines are measured by ELISA or multiplex assays.
- Study of anti-inflammatory capacity measuring cytokines produced in murine splenic lymphocytes. Primary cell cultures are treated with inflammatory agents and cytokines are measured by ELISA or multiplex assays.



Neuron Bio offers a broad portfolio of protocols for different evaluations such as eADME studies (*in silico* and *in vitro* models) or cell models (toxicity and efficacy)

Neuron Bio contributes to understanding and comprehension of numerous diseases by using the Multiplex technology through the study of specific biomarkers in patients

Antioxidant Cellular Capacity

- Study of antioxidant capacity in a cell model treated with a pro-oxidant drugs and measuring Reactive Oxygen Especies (ROS) production by a fluorescent probe assay.

Mechanism-Based Cellular Models

Alzheimer´s Disease Cell Model

- Using cell models carrying the APP (amyloid β A4 precursor protein) wild-type and Swedish-type mutant variants.

Inhibition of Acetylcholinesterase Activity

- Study of acetylcholinesterase activity measuring the inhibition by a spectrophotometric assay in cell lines.

Hypolipidemic Capacity

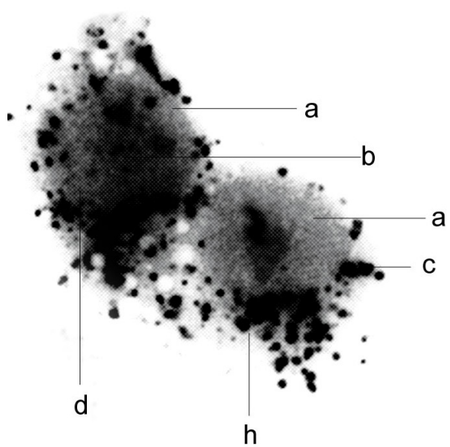
- Study of hypolipidemic capacity measuring cholesterol and triglycerides by a fluorescent probe assay in a hepatic cell lines.
- HMG-CoA Reductase (3-hydroxy-3-methylglutaryl-CoA reductase) activity *in vitro*. HMGCR is the rate-controlling enzyme of the mevalonate pathway.

Effect on Neurite Outgrowth

- Neuroblastoma differentiated cells are used to analyze the effect of the molecules in neurite outgrowth: (a) Study of toxicity in differentiated neuronal cells, (b) Analysis of morphometric parameters and (c) Quantification of neurite number, sum length, mean length and maximum length.

Neuronal Plasticity Gene Expression

- Differentiated human neuronal cells are used for neuronal plasticity assay. Neuronal plasticity gene expression is analyzed by qRT-PCR. Study of modulation of genes related to neuronal survival, synaptic plasticity, neurodegenerative processes and other neuronal functions after treatments with test compounds.



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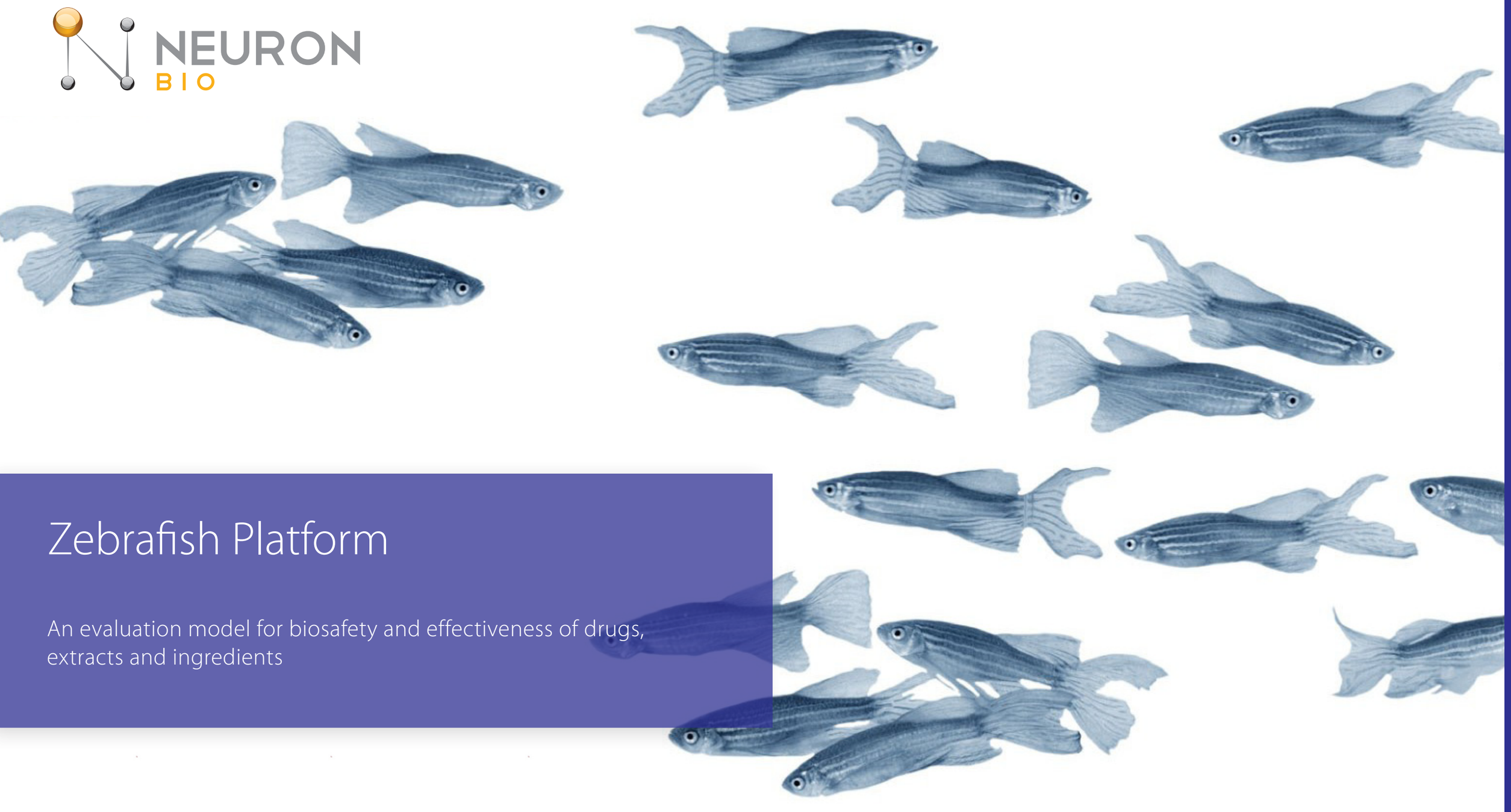


Zebrafish Platform

An evaluation model for biosafety and effectiveness of drugs, extracts and ingredients

Neuron Bio has developed a range of technologies based in the use of zebrafish to boost the development process of products of our clients. Thus, the zebrafish provides scientific evidence on the best products under development as a previous stage to their validation in mammals. Costs and development times are much more reduced in comparison with the rodent model

Toxicology

Fish Embryotoxicity (FET) Test

Analysis of the biosafety by measuring toxicological effects in embryo zebrafish model based on OCDE TG 236. Endpoints: (a) Lethal: coagulated embryos, lack of somite formation, non-detachment of the tail, heart rate alteration; (b) Sub-lethal: non-spontaneous movements, depigmentation, formation of edemas, blood coagulation; (c) Teratogenic: malformation of organs, scoliosis, general growth retardation; (d) Tox Parameters: half lethal concentration (LC50); no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC).

Ototoxicity

Ototoxicity studies on neuromasts of the lateral line of larvae zebrafish (detected by fluorescence microscopy) caused by any kind of compound.

Ocular Toxicity

Use of zebrafish embryo/larvae/adult to predict adverse visual effects in early drug safety assessment (by histology&histopathology and immunohistochemistry).

Limit Test

Using the procedures described in the OECD TG 203, a limit test may be performed at 100 mg/l in order to demonstrate that the LC50 is greater than this concentration. If any mortality occurs, a full study should be conducted. If sublethal effects are observed, these should be recorded.

Single Dose Toxicity

Analysis of the biosafety by measuring toxicological effects of a single dose of a compound in adult zebrafish based on OECD TG 203. Endpoints: (a) Mortality; (b) Sub-lethal effects: loss of equilibrium, erratic swimming behaviour, abnormal respiratory function, alteration of pigmentation.

Fish Acute Toxicity Test

Analysis of the biosafety by measuring toxicological effects in adult zebrafish based on OECD TG 203. Endpoints: (a) Mortality; (b) Kaplan-Meier analysis; (c) Sub-lethal effects: loss of equilibrium, erratic swimming behaviour, abnormal respiratory function, alteration of pigmentation; (d) Tox Parameters: half lethal concentration (LC50); no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) values.

Efficacy

Neuroprotection in Zebrafish Embryo/Larva

Analysis of protective effect against neuronal damage caused by neurotoxin.

Central Nervous System Development

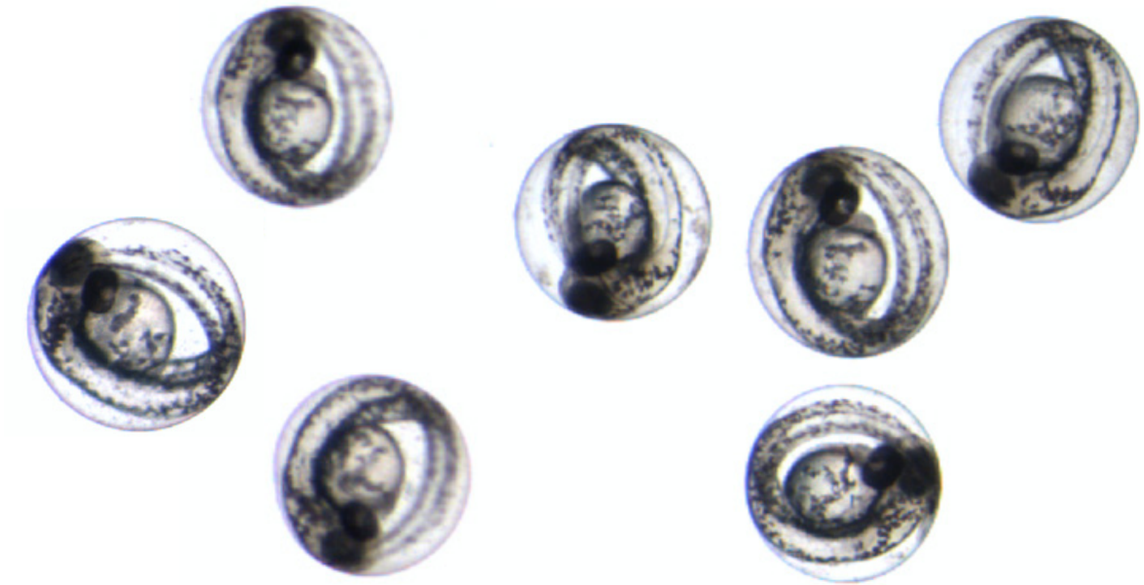
Effect of compounds in CNS development. Endpoints: (a) Axonal growth, (b) Motoneurons detection and (c) Specific neuronal biomarkers.

Neuroprotection in Adult Zebrafish

Study of α-, β- and γ-secretase activities in zebrafish brain measured by fluorescent specific substrates.

Epilepsy Model

Study of compounds in an epilepsy model measuring behaviour seizure-stage score in adult zebrafish after kainate injection. Endpoint: (a) Mortality; (b) Kaplan-Meier analysis; (c) Latency: time of seizure onset; (d) Racine's scale: seizure



An alternative to mice with the goal of reducing the use of mammals in research

One of the most important advantages of this animal model is that it is excluded from the animal testing regulation because it is not considered as a vertebrate organism until 6 days after birth, thus allowing experimentation with them, avoiding long bureaucratic periods related to animal testing

score; (e) Status epilepticus (SE): % of animals with SE; (f) Seizures: % of animals with seizures.

Locomotor Activity and Anxiety

Open Field. Endpoints: (a) Latency; (b) Swimming velocity; (c) Resting time; (d) Swimming distance; (e) Thigmotaxis.

Cognitive Status and Spatial Memory

T-Maze. Endpoints: (a) Latency; (b) Swimming activity; (c) Total time in enriched chamber.

Screening of Antipsychotics in Adult Zebrafish

Investigation of potential antipsychotic effects. The blockade of the hyperactivity induce by a NMDA receptor antagonist is predictive of antipsychotic-like efficacy.

Protection Against Ototoxicity Phenomena

Protective effect of a compound against ototoxicity caused by aminoglycoside antibiotics on neuromasts of the lateral line of larvae zebrafish (detected by fluorescence microscopy).

Tools

Histology/Histopathology

Cytoarchitecture (H&E).

Immunohistochemistry/Immunofluorescence

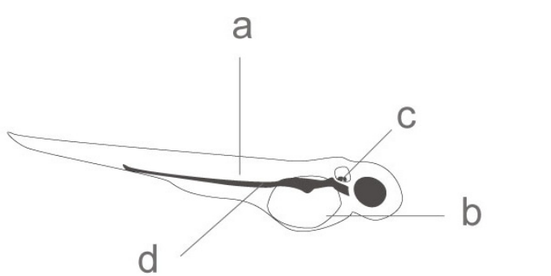
Neuronal markers (Acetylated α-tubulin, TH, Isl 1/2), astrogliosis (GFAP)…on whole mount (embryo/larva) and histological samples (adult).

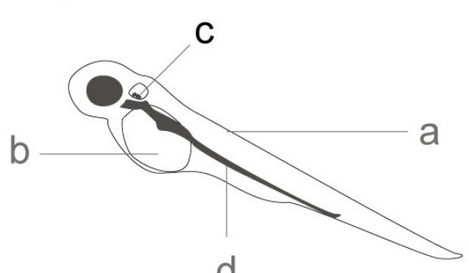
Western Blot

Brain neuronal markers.

Behaviour

- Cognitive status: Spatial memory (T-maze).
- Global motor activity: Global activity (Open-Field).





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Rodent Platform

The rodent platform will definitely help you to decide go/no-go in the development of your products

Rodent models are integral to our understanding of the cellular and molecular pathogenesis of human diseases and disorders. They allow us to study the function of genes in a living animal to develop better strategies for diagnosis, treatment and prevention of different kinds of cancer, obesity, heart disease, diabetes, neurodegenerative diseases, etc; offering a biological context, in which drugs and other therapies can be developed and tested

Toxicology

Non-Regulatory Toxicology (in Mice and Rats)

Toxicological evaluation in mice and rats by using several routes (oral gavage, intraperitoneal, intravenous, diet administration, etc), different doses and schedules of administration. Type of studies: Acute studies to determine LD50; Dose Range inding (DRF) studies with MTD determination; 14-28 day-studies to characterize the toxicological profile. Endpoints:

- *In vivo*: (a) Health status (including a veterinary report); (b) Weight evolution; (c) Hemogram, (d) Biochemical determinations, (e) Diet and beverage intake; (f) Mortality.
- *Post-mortem*: (a) Necropsy; (b) Hemogram; (c) Biochemical determinations; (d) Histopathological analysis of target organs.

Efficacy

Acute and Chronic Neural Death Model (in Mice)

Search of new neuroprotectants by the study of acute/chronic neurodegeneration by systemic administration of a neurotoxin, causing seizures, excitotoxicity, oxidative damage, neuritic distrophy, neuroinflammation, and apoptosis in the hippocampus and other limbic structures of the brain cortex. Endpoints: (a) Mortality: Kaplan-Meier analysis; (b) Anticonvulsant activity: seizure score, latency, % of animals with seizures, % of animals with status epilepticus; (c) Neuropathological analysis; (d) Immunohistochemical analysis.

Dopaminergic Neural Death Model (in Mice)

Study of acute neurodegeneration by systemic administration of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), causing locomotor alterations, neuron death by apoptosis and gliosis in the *substancia nigra* and *striatum*. Endpoints: (a) Mortality: Kaplan-Meier analysis; (b) Global motor activity; (c) Neuropathological analysis; (d) Immunohistochemical analysis.

Hypercholesterolemia (in Mice , Rats and Guinea Pig)

Study of hypercholesterolemia in induced (pharmacologically or by the diet) mouse models. Acute, sub-chronic or chronic studies can be developed by using several mouse models. Endpoints: (a) Biochemistry; (b) Macroscopic evaluation: weight evolution, fat accumulation.

Atherosclerosis (in Mice and Rats)

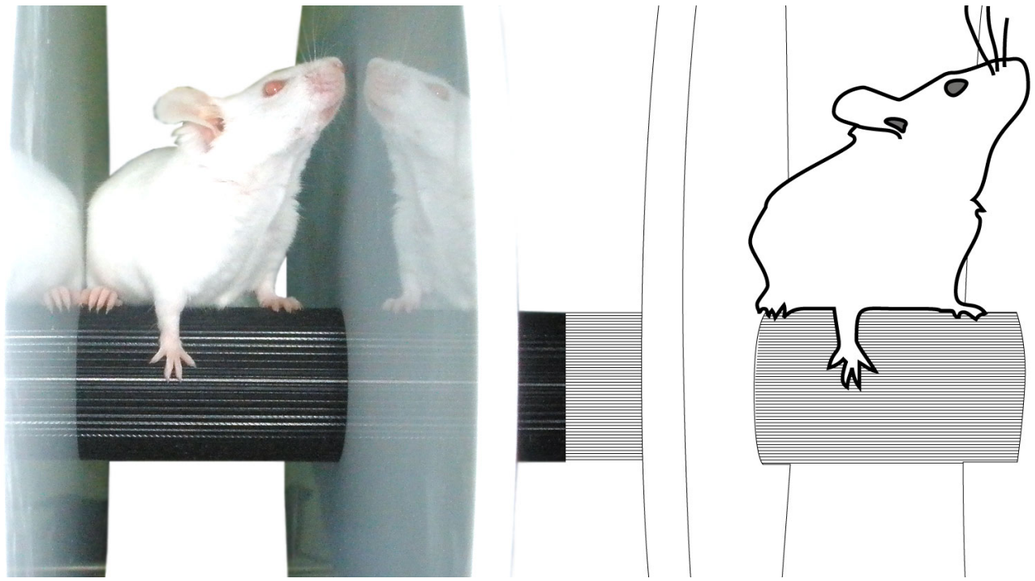
Study of atherosclerosis in hypercholesterolemic rodent models. Endpoints: (a) Biochemistry; (b) Neutrophil infiltration; (c) Histopathology: lipid content (Sudan), cytoarchitecture (H&E) and collagen content (Van Gieson).

Pharmacokinetics

Pharmacokinetics (in Mice and Rats)

Determination of PK parameters in mice and rats by using several routes (oral gavage, intraperitoneal, intravenous, diet administration, etc). Several organs are analyzed (plasma, brain, etc), and different analytical methods are used (UPLC-MS, GC-Fid). Endpoints:

- C_{max} (maximum concentration reached).
- AUC (area under the curve).
- T_{max} (time of the C_{max}).
- $T_{1/2}$ (elimination half-life).
- V_D (volume of distribution).
- C_L (clearance).



Studies are conducted in mouse and rat models according to the guidelines of regulatory agencies

Animal experimentation is conducted under the supervision of the company own animal experimentation board and is subject to an official veterinarian report according to recent Spanish legislation RD 53/2013

Tools

Histology/Histopathology

Cytoarchitecture (H&E), neurodegeneration (FJB), apoptosis (Acridine Orange).

Immunohistochemistry/Immunofluorescence

Apoptosis (TUNEL), Neuronal loss/dendritic atrophy (MAP2), astrogliosis (GFAP), microgliosis (Iba-1), lipid peroxidation (HNE), neuronal markers (NeuN, TH), oligodendrocytes (OSPA), etc.

Western Blot

Brain neuronal markers: NeuN, Aβ, Tau, Tau-p, etc.

Behaviour

- Cognitive status: Spatial memory (Morris Water Maze and Y-maze), Temporal memory (Passive Avoidance Test), Episodic-like memory (Integrated Memory Test).
- Global motor activity: Strength (Grip-Strength), Global activity (Open-Field), Motor coordination (Rota-Rod test).

Molecular Imaging

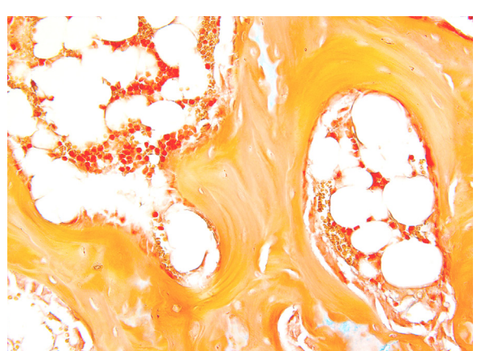
Using of bioluminescence to study neuroinflammation.

Biomarkers

Peripheral and brain inflammatory markers: ELISA analysis (IL-6, IL-1b, etc) and Milliplex® Technology (up to 32 markers, mainly cytokines).

Biochemical Determinations (In Plasma and Urine)

- Biochemistry: plasma cholesterol fractions (total, free, esterified, LDL-c, HDL-c), lipoproteins (ApoA, ApoB), triglycerides, lipid peroxidation, and liver studies (triglycerides, cholesterol and fat content), AST, ALT, CK, creatinine, glucose, triglyceride, cholesterol, LDL-c, HDL-c, proteins, etc.
- Hemogram.

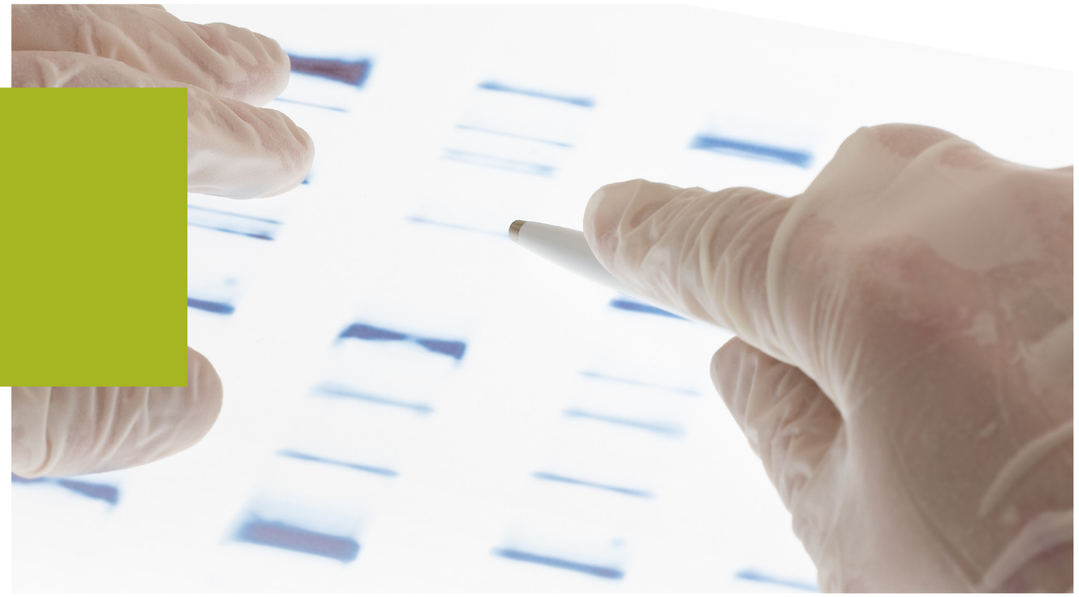


NEURON BIO

Transgenic Model Platform

Integral tailor-made solutions to generate your own transgenic animal for your research purposes

You can contract us to perform the entire transgenic project from design and DNA vector construction to phenotyping and maintenance of transgenic line, as well as milestone steps for partial services



DNA Vector Construction

The construction of targeting vectors is a pre-requisite for the generation of genetically engineered animals and establishment of a transgenic cell lines. Our molecular biology platform provides design, construction and modification of different DNA vectors (plasmids, BACs and YACs) for different research aims such as transgene expression or genome editing (Knockout and Knockin).

Animal Models

Mice and Zebrafish

Generation

Neuron Bio generates your own transgenic/knockout/knockin mouse or zebrafish by microinjection or electroporation of different constructs into fertilized oocytes/eggs.

Services:

- DNA purification.
- DNA linearization.
- Screening.
- Genotyping.
- Breeding, feeding , establishment and maintenance of founder and F1 animals.

Transgenic mice and zebrafish provide excellent models of human diseases. They allow us to evaluate the effects of single altered genes in the context of the whole organism and provide tremendous insight into gene function. However, they can provide research results that are frequently unexpected, confusing or simply uninformative. A broad phenotypic characterization of emerging mouse models will add important value and greatly enhance their utility. For this reason, Neuron Bio is committed to providing comprehensive phenotyping services for both species.

Phenotyping

Services:

- Genotipic analysis of founders.
- Analysis of transgene expression (Western Blot & RT-PCR).
- Behavioural and cognitive phenotyping.
- Histology & histopathology, inmunohistochemistry (IHQ).
- Imaging techniques (bioluminescence, fluorescence…).
- Multiplex detection of immunology.

Mammalian Stable Cell Lines

Human and Mouse Lines

Stable cell lines are an essential tool for drug screening, gene functional studies and other applications. They can grow continuously over a prolonged period of time and stably carry a genetic modification or express a transgene without significant changes in expression levels.

Neuron Bio offers state-of-the-art services for establishing stable cell lines for genome editing, gene knockdown or protein overexpression, that meet your specific research needs.

Contact us

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Neuron Bio online

To keep up to date with all of Neuron Bio activities please visit **neuronbio.com**

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