

OTOTOX ASSAY

Biobide is a biotechnology company offering drug discovery services to Pharma, Biotech, Chemical, Cosmetic and Nutraceutical companies. Our services are based on the **zebrafish model** and the capacity to offer highly efficient tailor made assays.

The zebrafish embryo is an emerging model due to its inherent properties (ease to manipulate, external fertilization and transparency) together with the need to apply the 3Rs. This model has a high genetic homology with humans (over 85%) as well as important parallels in organogenesis and functional mechanisms.

Biobide has developed a method to detect the capability of a compound to induce ototoxicity as part of safety pharmacology studies. Although the detailed structure of the inner ear varies among vertebrates, all species rely on hair cells to detect both vestibular and auditory sensory stimulation. Hair cells convert this mechanical force into electrical impulses that are carried by the auditory nerves to the brain. In addition to the inner ear, zebrafish have superficial mechano-sensory organs, called neuromasts, present on the lateral line along the head and body that detect vibrations in the surrounding environment at frequencies from 50 to 200 Hz. Each neuromast contains a ring of supporting cells which surround a central cluster of sensory hair cells. Hair cells in zebrafish neuromasts are similar in structure and function to the inner ear hair cells in mammals. Similar to the processes involved in degeneration of hair cells in the organ of Corti in mammals, neuromast hair cells in zebrafish have been shown to undergo programmed cell death during normal turnover and after injury with known ototoxic drugs, validating the use of this organism as a tool for ototoxicity screening.

The assay is performed under Good Laboratory Practice (GLP) environment.

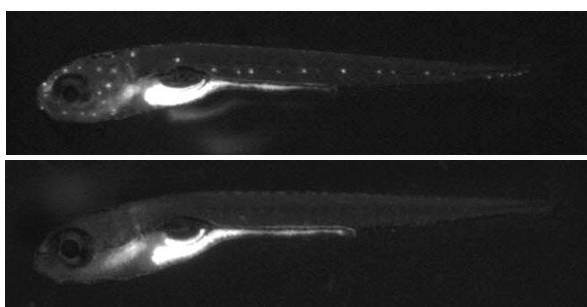


Figure 1. Fluorescent image of 6 dpf (days post-fertilization). (a) Neuromasts stained with DASPEI located along the lateral line as discrete white spots in WT non-treated mice. (b) Neuromasts are not detected after the treatment with ototoxic compounds.

METHOD DESCRIPTION

Experimental model: Zebrafish (*Dario rerio*) wild-type embryos obtained from crossing adult zebrafish under strict environmental conditions of temperature and photoperiod are used.

Treatment: 5 days post fertilization (dpf) embryos are dispensed in 24 well-plates, 5 embryos per well, 2 well/concentration and 5 concentrations.

Incubation: test plate is incubated for 24 h.

Data collection: after 24 h of treatment, embryos are stained with DASPEI. DASPEI staining is used in order to visualize and analyze the number of neuromasts present in the lateral line of zebrafish embryos after a compound treatment.

After washing, embryos are anesthetised with tricaine and positioned in methyl-cellulose in order to take images.

Analysis: pictures obtained are analysed by counting the number of neuromasts present in the lateral line of the embryo (Figure 1, 2).

Subsequently, statistical analysis is performed and data are interpreted by the Study Director.

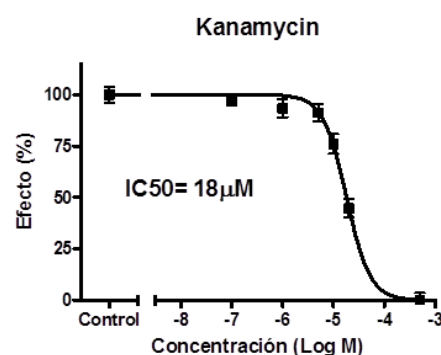


Figure 2. IC50 curve

VALIDATION RESULTS

To validate the Ototox Assay, the effects of five known ototoxic compounds and a negative control have been studied in zebrafish embryos (Table 1, 2; Figure 3).

Table 1. Concentrations inducing ototoxicity in Zebrafish

Compound	Concentration (μM)									
	0.1	1	5	10	20-25	50	75	100	200	500
Neomycin	Not tested	No effect	Acute effect	Acute effect	Acute effect	Acute effect	Acute effect	Acute effect	Toxic/lethal	Acute effect
Kanamycin	No effect	No effect	Moderate effect	Acute effect	Acute effect	Acute effect	Acute effect	Acute effect	Acute effect	Acute effect
Cisplatin	Not tested	Not tested	Not tested	Moderate effect	Acute effect	Acute effect	Acute effect	Acute effect	Acute effect	Acute effect
Quinine	Not tested	Not tested	Not tested	No effect	Moderate effect	Moderate effect	Moderate effect	Moderate effect	Acute effect	Acute effect
Ethacrynic	Not tested	Not tested	Not tested	Moderate effect	Moderate effect	Moderate effect	Toxic/lethal	Toxic/lethal	Toxic/lethal	Acute effect
Ampicillin	Not tested	Not tested	Not tested	No effect	No effect	No effect	No effect	No effect	No effect	No effect

Not tested
 No effect
 Moderate effect
 Acute effect
 Toxic/lethal

*Moderate effect: the number of neuromasts present after treatment is higher than half of the number found in control embryos.

**Acute effect: the number of neuromasts present after treatment is lower than half of the number found in control embryos.

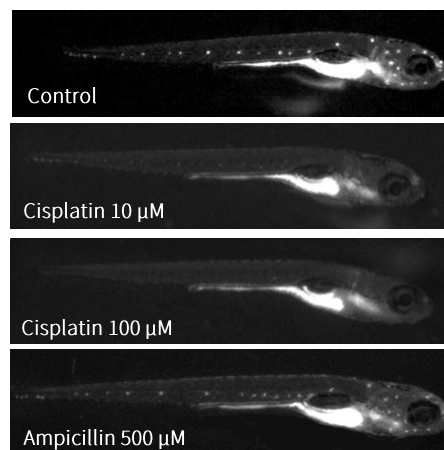


Figure 3. Examples of treated zebrafish embryos. In control embryos, neuromasts are easily detected in the lateral line. Nevertheless, embryos treated with the lowest concentration of cisplatin show a decrease in the intensity of the neuromasts present, although many of them are still detected. With the highest concentration of cisplatin, almost all neuromast disappear. In contrast, embryos treated with ampicillin are indistinguishable from control ones.

Table 2. Ototoxicity detected in different systems

Compound	Ototoxicity in			
	Zebrafish	Cells	Animal models	Humans
Neomycin	Yes (1-10 μM)	Yes(100-600 μM)	Yes	Yes
Kanamycin	Yes (5-20 μM)	ND	Yes	Yes
Cisplatin	Yes (10-50 μM)	Yes(50 μM -10mM)	Yes	Yes
Quinine	Yes (20-200 μM)	Yes (50 μM -1.5mM)	Yes	Yes
Ethacrynic Acid	Yes 810-50 μM)	ND	Yes	Yes
Ampicillin	No	ND	No	No

- ✓ Zebrafish embryos can be used to analyse the ototoxicity induced by a compound.
- ✓ Zebrafish embryos can be used in drug discovery to provide early screening assays which comply with 3R principle.