



Zebrafish experts
in **Early Discovery and**
Development services

● Your
Zebrafish
Partner

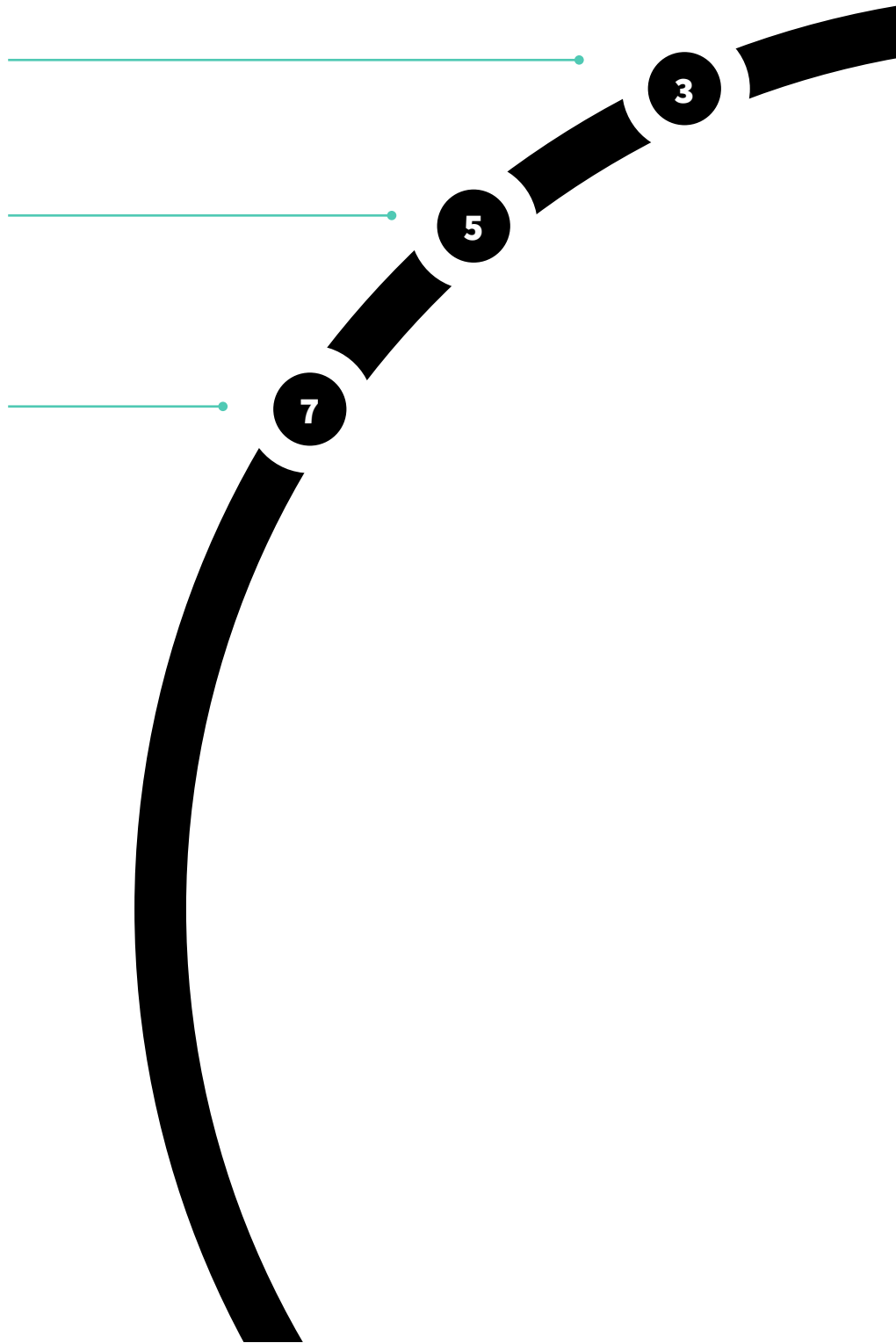
INDEX

1. ABOUT BIOBIDE

2. ADVANTAGES OF ZEBRAFISH IN R&D

3. VALIDATED ASSAYS

- A. TARGET VALIDATION
- B. ZEBRAFISH MODEL GENERATION
- C. EFFICACY ASSAYS
- D. TOXICITY ASSAYS
- E. ECOTOXICITY ASSAYS



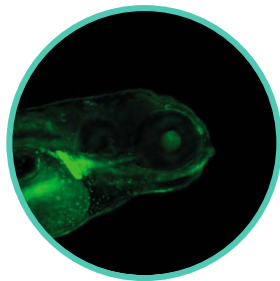


ABOUT BIOBIDE

Biobide is a Biotech company with **more than 16 years of experience** offering Zebrafish Services to Pharmaceutical, Biotech, Chemical and Cosmetic companies under **Good Laboratory Practices (GLPs)**.

Our main aim is to accelerate our Clients' R&D process by minimizing risks using **zebrafish alternative model**, adding value mainly in early phases through disease model generation, toxicity and efficacy assays.

OUR VALUES



KNOW-HOW

We speak of **precision, technique, and control** because we've mastered the zebrafish technology and screening process.



AUDACITY

Our ability to **listen** and our tenacity to find the **optimal solutions** allows us to understand the needs of our customers.



VITALITY

We like what we do and we are committed to promoting **innovation** in biotechnology and in the zebrafish screening process.



EFFICIENCY

Efficiency is the key to **success** with our customers: because we are **tenacious, meticulous and resolute**.



ADVANTAGES OF ZEBRAFISH IN R&D

Relevance of the zebrafish model for modeling human diseases:

- ✓ High genetic homology with humans.
- ✓ Suitable for efficacy, and general and organ specific toxicity screenings.

Easy manipulation for assay development:

- ✓ Small size (similar to cells).
- ✓ Transparent embryos.
- ✓ High productivity: 100-300 eggs/week & couple.
- ✓ Fast development/organogenesis.
- ✓ Direct administration of compounds to the medium.
- ✓ Suitable for automation, integration and image analysis for phenotypic screenings.
- ✓ Easy genetic manipulation.

Other main advantages:

- ✓ High Cost and Time efficiency.
- ✓ Fewer ethical impediments (3 Rs).
- ✓ Highly informative results.





VALIDATED ASSAYS

Services **tailored** to your needs

TARGET VALIDATION

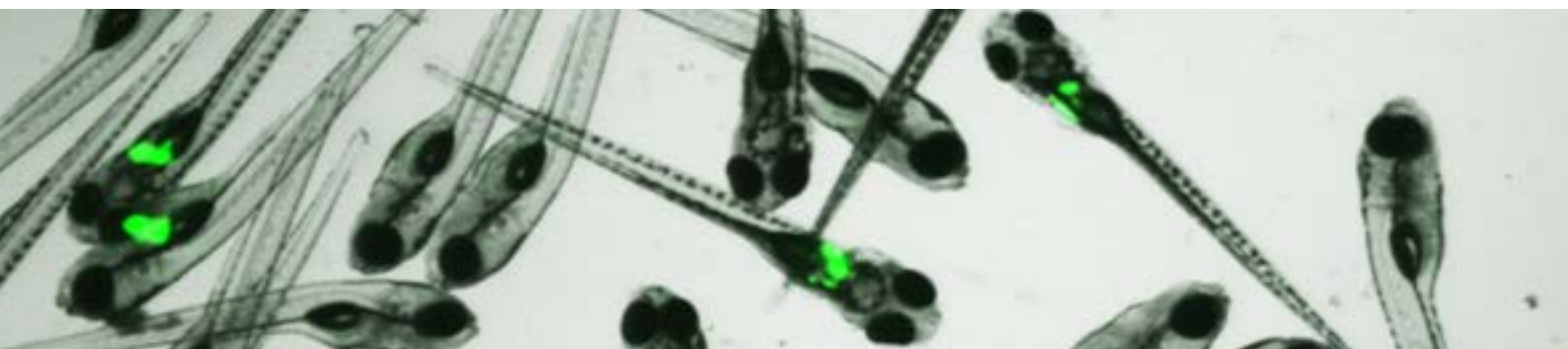
Target identification and validation through:

- ✓ Gene silencing with **morpholino antisense oligonucleotides** – *gene knock-down* –.
- ✓ Transient mutations with **CRISPR/Cas13**.

ZEBRAFISH MODEL GENERATION

Development of zebrafish models*:

- ✓ Chemically induced models.
- ✓ Transgenesis (**Tol2 kit**).
- ✓ Generation of mutants:
 - ✓ **CRISPR/Cas9**
 - ✓ **TALENS**
- ✓ Xenograft models: by human cells injection.



EFFICACY ASSAYS

Zebrafish **disease models** available for different therapeutic areas*:

Central Nervous System

- ✓ Evaluation of **behaviour alterations** in embryos or adult fish: neurodegenerative diseases (AD, PD...), mood disorders (anxiety, stress, depression...), sleeping disorders, addiction, reward...

Rare diseases

- ✓ **Neuromuscular diseases:** ALS, Dravet, DMD...
- ✓ **Others:** Fragile X syndrome, cystic fibrosis...

Others

- ✓ **Infectious disease models:** bacteria, virus or fungus...
- ✓ **Efficacy assays for cosmetics:** regeneration, "whitening"...

Oncology

- ✓ Angiogenesis inhibition assay.
- ✓ Zebrafish tumoral models: hepatocarcinoma, melanoma, p53...
- ✓ Immune-oncology: Innate and acquired immune responses evaluation in zebrafish tumoral models.

Cardiovascular and Metabolic diseases

- ✓ Angiogenesis inhibition / proliferation.
- ✓ Metabolic alterations and enzyme expressions.
- ✓ Obesity, Cholesterol, Diabetes...
- ✓ Enzyme alterations.

*Please, contact us regarding the disease model of your interest

GENERAL TOXICITY



- ✓ Based on OECD 236.
- ✓ **Mortality measure** at 2 and/or 4 dpf* as:
 - Coagulated embryos.
 - Lack of somites.
 - Lack of heartbeat.
 - Non-detachment of the tail.



- ✓ EC50, LC50, Teratogenic Index (TI) and NOAEL.
- ✓ **>10 morphological endpoints** analyzed at 2 and/or 4 dpf:
 - Craneofacial malformation.
 - Malformation of the otic vesicle.
 - Malformation of the heart.
 - Deformed body shape.
 - Malformation of the caudal fin.
 - Yolk deformation.
 - Necrotic tissues.
 - Hatching.

ORGAN SPECIFIC TOXICITY



- ✓ TG zebrafish expressing GFP in the heart.
- ✓ Biobide's Cardio v3.0.0.5 software.
- ✓ **Endpoints** at 2 and 3 dpf:
 - Heart rate.
 - Presence of arrhythmia.
 - Absence of heartbeat (death/fibrillation).



- ✓ Locomotor activity/photomotor response tracked alternating photoperiods (dark-light).
- ✓ DanioVision System (Noldus).
- ✓ **>10 parameters** measured at 5 dpf based on distance, velocity, movement duration and frequency.
- ✓ Also in **adult zebrafish**.

dpf: days post fertilization*



- ✓ TG zebrafish with fluorescent liver.
- ✓ **Acute Hepatic Toxicity at 5 dpf***:
 - Liver opacity assessment.
 - Liver's area and fluorescent intensity evaluation.
- ✓ **Hepatic steatosis.**
- ✓ **Expression analysis of genes** related to hepatic metabolism.
- ✓ **Histopathology.**



- ✓ Transgenic Zebrafish with fluorescent immune cells.
- ✓ **Innate immune system:** quantification leukocyte population, infiltration of leukocytes.
- ✓ **Adaptive immune system:** lymphopoiesis, susceptibility to ablative agents.
- ✓ Immune response **gene expression.**



- ✓ DASPEI staining to visualize and analyze the number of **neuromasts** after 24 hours of treatment.



- ✓ **Kidney alterations** (morphological changes):
 - Pronephros structure.
- ✓ **Functional assays:**
 - Glomerular function.
 - Tubular function (clearance).



- ✓ **Fluorescence assay:** TG zebrafish with fluorescence in the thyroid gland
 - Benchmark Concentration (BMC) and Thyroid Disrupting Index (TDI) are calculated.
- ✓ Thyroid related **gene expression** by qPCR.

dpf: days post fertilization*

ECOTOXICITY ASSAYS

Acute Toxicity & Endocrine Disruption Screening Assays for **Aquatic Toxicity Assessment:**

1. ALGA:



Microplate Alga Growth-Inhibition Test

- ✓ Scaled-down version of the standard OECD guideline 201 carried out in microplate format. Requires low test volumes, and it is suitable for **High-Throughput Screening purposes.**

2. DAPHNIA:



Daphnia magna Immobilization Test

- ✓ Acute toxicity assay on Daphnia defined by the **OECD Test No. 202: Daphnia sp. Acute Immobilization Test.**

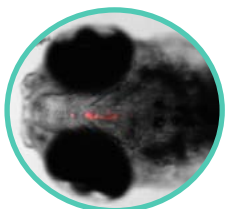
3. ZF ECOTOXICITY ASSAYS:



Acute Toxicity Screening Assay in Zebrafish Embryo

- ✓ **Simplified Fish Embryo Toxicity (FET) Test:** a fast and cost/time-effective screening assay to assess the aquatic toxicity of chemicals in zebrafish embryos.

Endocrine Disruption (ED) Screening Assay:



- ✓ Screening assays for identification of potential ED substances in zebrafish transgenic embryos:

- **Thyroid pathway**
- **Estrogen pathway**
- **Androgen pathway**


Download our
TECHNICAL SHEETS
through our QR code:



BIOBIDE USA

One Broadway, 14th floor


Cambridge, MA 02142

 +1 617 659 0295

BIOBIDE SPAIN

Gipuzkoa Scientific & Technological Park

Pº Mikeletegi 56, 20009 San Sebastian

 +34 943 309 360



info@biobide.com

www.biobide.com