

# Viability assay with fish gill cell line to assess acute toxicity

*Commonly used acronym: RTgill-W1 cell line assay*

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## Organisation

**Name of the organisation** Vlaamse Instelling voor Technologisch Onderzoek (VITO)

**Department** Health

**Country** Belgium

**Geographical Area** Flemish Region

## Partners and collaborations

Swiss Federal Institute of Aquatic Science and Technology (EAWAG)

## SCOPE OF THE METHOD

<b>The Method relates to</b>	Environment
<b>The Method is situated in</b>	Translational - Applied Research
<b>Type of method</b>	In vitro - Ex vivo
<b>Species from which cells/tissues/organs are derived</b>	Rainbow trout, <i>Oncorhynchus mykiss</i>
<b>Type of cells/tissues/organs</b>	Gill tissue

## DESCRIPTION

### Method keywords

cell viability test

fish gill cell line  
cell metabolic activity  
lysosomal membrane integrity  
cell membrane integrity

### **Scientific area keywords**

fish acute toxicity  
chemical exposure

### **Method description**

The rainbow trout gill cell line assay quantifies cell viability using fluorescent measurements for metabolic activity (Alamar Blue, AB), cell membrane integrity (5-CarboxyFluorescein DiAcetate AcetoxyMethyl ester, CFDA-AM) and lysosomal membrane integrity (Neutral Red, NR). Chemicals are added to confluent RTgill-W1 cell monolayers in 24-well plates with L-15/ex medium (a simplified version of L-15 cell culture medium without serum). Cells are incubated for 24 hours in the incubator (19°C, normal atmosphere, in the dark). At the end of the exposure, cell viability measurements are performed with 3 fluorescent indicator dyes on the same set of exposed cells.

### **Lab equipment**

Laminar flow ;  
Incubator (room temperature, no CO2) ;  
Microplate reader for fluorescence detection.

### **Method status**

History of use  
Internally validated  
Published in peer reviewed journal

## **PROS, CONS & FUTURE POTENTIAL**

### **Advantages**

Cell line model with limited requirements ;  
Robust assay: repeatability and reproducibility is shown through inter- and

intralaboratory studies ;  
Alternative model to predict fish acute toxicity.

## Challenges

Exposure of chemicals (bioavailability).

## REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

### References

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Tanneberger, K., Knoebel, M., Busser, F. J. M., Sinnige, T. L., Hermens, J. L. M., and Schirmer, K. (2013). Predicting fish acute toxicity using a fish gill cell line-based toxicity assay. *Environ. Sci. Technol.* 47, 1110–1119.

ISO 21115:2019. Water quality — Determination of acute toxicity of water samples and chemicals to a fish gill cell line (RTgill-W1).

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