

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

*Commonly used acronym: RTgill-W1 cell line assay*  
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## SCOPE OF THE METHOD

<b>Method relates to</b>	Environment
<b>Method is situated in</b>	Translational - Applied Research
<b>Type of method</b>	In vitro - Ex vivo
<b>This method makes use of</b>	Animal derived cells / tissues / organs
<b>Species from which cells/tissues/organs are derived</b>	Rainbow trout, Oncorhynchus mykiss
<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 CO<sub>2</sub>) ;  
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

Fischer, M ; Belanger, SE ; Berckmans, P ; Bernhard, MJ ; Blaha, L ; Schmid, DEC ; Dyer, SD ; Haupt, T ; Hermens, JLM ; Hultman, MT ; Laue, H ; Lillicrap, A ; Minarikova, M ; Natsch, A ; Novak, J ; Sinnige, TL ; Tollefsen, KE ; von Niederhausern, V ; Witters, H ; Zupanic, A ; & K. Schirmer (2019). Repeatability and reproducibility of the RTgill-W1 cell line assay for predicting fish acute toxicity. *Toxicological Sciences*, 169 (2), 353-3640.

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).

### Associated documents

## PARTNERS AND COLLABORATIONS

### Organisation

**Name of the organisation** VITO

**Department** Health

**Country** Belgium

**Geographical Area** Flemish Region

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