

# ISO 21115:2019-04 (E)

## Water quality - Determination of acute toxicity of water samples and chemicals to a fish gill cell line (RTgill-W1)

---

### Contents

Page

Foreword	iv
Introduction	v
<b>1 Scope</b>	<b>1</b>
<b>2 Normative references</b>	<b>1</b>
<b>3 Terms and definitions</b>	<b>1</b>
<b>4 Principle</b>	<b>2</b>
4.1 Cell viability assay	2
4.2 Key differences in water sample and chemical testing procedure	3
<b>5 Interferences</b>	<b>3</b>
5.1 Matrix effects by effluent samples	3
5.2 Interferences of water constituents or chemicals with fluorescent dye assays	4
<b>6 Reagents</b>	<b>4</b>
6.1 General	4
6.2 Ready-for-use purchased reagents	4
6.3 Freshly prepared solutions	6
<b>7 Apparatus and material</b>	<b>8</b>
7.1 General equipment	8
7.2 Whole-water sample/ex preparation	9
7.3 Cell seeding and plate dosing	9
7.4 Preparation/dosing of stock solutions	9
7.5 Sampling for chemical analysis	10
7.6 Detection of cytotoxicity	10
<b>8 Procedure</b>	<b>10</b>
8.1 Cell line used	10
8.2 Seeding cells into 24-well plates	10
8.2.1 General	10
8.2.2 Preparation of working materials and solutions for cell seeding	10
8.2.3 Seeding cells: step-by-step performance	11
8.3 Cell exposure	12
8.3.1 General	12
8.3.2 Exposure of cells to water samples	13
8.3.3 Exposure of cells to chemicals	18
8.4 Determination of cytotoxicity	21
8.4.1 General	21
8.4.2 Visual control of cell damage	22
8.4.3 Preparation of working materials and solutions for cytotoxicity measurement	23
8.4.4 Measuring cytotoxicity — Step-by-step performance	23
8.4.5 Parameters for the fluorescence measurement	24
8.4.6 Chemical analyses of the test samples	24
<b>9 Preparation and expression of results</b>	<b>24</b>
<b>10 Validity criteria of the test</b>	<b>25</b>
10.1 No cells-control wells	25
10.2 Positive control in L-15/ex	25
10.3 Positive control in w-ws/ex	26
10.4 Solvent control	26

<b>Annex A (informative) Routine cell culture of RTgill-W1</b> .....	<b>27</b>
<b>Annex B (informative) Performance of the RTgill-W1 cell line assay</b> .....	<b>32</b>
<b>Bibliography</b> .....	<b>38</b>