DIN EN ISO 19040-3:2023-12 (E)

Water quality - Determination of the estrogenic potential of water and waste water - Part 3: Invitro human cell-based reporter gene assay (ISO 19040-3:2018)

Contents

Page

European foreword ... 4

Foreword ... 5

1 Scope ... **6**

2 Normative references ... **6**

3 Terms and definitions ... **6**

4 Interferences ... **9**

5 Principle ... **9**

6 Apparatus and materials ... **9**

7 Reagents, cells and media ... **10**

8 Sampling and samples ... 14
8.1 General ... 14
8.2 Bottles and material for sampling ... 14
8.3 Bottles and material pre-cleaning ... 14
8.4 Sampling procedure ... 14
8.5 Transport of samples ... 14
8.6 Pretreatment of sample ... 15
8.7 Storage of samples ... 15

9 Procedure ... **15**
9.1 Cell culture maintenance ... 15
9.1.1 Freezing cells ... 15
9.1.2 Starting a new cell culture ... 15
9.1.3 Culturing cells ... 16
9.2 Human cell reporter gene assay test procedure ... 16
9.2.1 Seeding the cells (day 1) ... 16
9.2.2 Preparation of the E2-reference (day 2) ... 16
9.2.3 Preparation of the sample dilutions (day 2) ... 17
9.2.4 Field blank ... 17
9.2.5 Exposing the cells (day 2) ... 17
9.2.6 Harvesting the cells (day 3) ... 18
9.2.7 Measurement of luminescence (day 3) ... 18
9.3 Data analysis ... 18
9.3.1 Calculation of the reporter gene induction ... 18
9.3.2 Calculation of the percentage of maximum response ... 19
9.3.3 Calculation of the dose-response curve ... 19

10 Validity criteria ... **19**
10.1 Validity criteria for the assay ... 19
10.2 Validity criteria for samples ... 20

11 Assessment criteria ... **20**

12 Test report ... **20**

Annex A (informative) **Settings of the luminometer** ... **21**

Annex B (informative) **Plate setup** ... **22**

Annex C (informative) **Bioassay characteristics and details** ... **23**

Annex D (informative) **Test set up for chemicals and extracts** ... **25**

Annex E (informative) **Preparation of dilution series** ... **27**

Annex F (informative) **Performance data** **28**
Annex G (informative) **Statistical assessment** **38**
Annex H (informative) **Calculation of 17β-estradiol equivalents** **39**
Annex I (informative) **Measurement of the lowest ineffective dilution (LID) of waste water — A simplified evaluation for testing of waste water** **41**
Bibliography **44**