

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