Table of Contents

10.0 Practical Considerations 2

10.1 Transferability of the BG1Luc ER TA 2

10.1.1 Facilities and Major Equipment 2

10.1.2 General Availability of Other Necessary Equipment and Supplies 2

10.1.3 BG-1Luc4E2 Cell Line 3

10.2 BG1Luc ER TA Training Considerations 3

10.3 Time and Cost Considerations 3

10.0 Practical Considerations

Several issues are taken into account when assessing the practicality of using an alternative to an existing test method. In addition to performance evaluations, assessments of the laboratory equipment and supplies needed to conduct the alternative test method, level of personnel training, labor costs, and the time required to complete the test method relative to the existing test method are necessary. The time, personnel cost, and effort required to conduct the proposed test method(s) must be considered to be reasonable when compared to the existing test method it is intended to replace. This section discusses the practical issues associated with using the BG1Luc ER TA for the determination of ER agonist and antagonist activity.

10.1 Transferability of the BG1Luc ER TA

Test method transferability addresses the ability of a method to be accurately and reliably performed by multiple laboratories (ICCVAM 2003), including those experienced in the particular type of procedure as well as laboratories with no prior experience. The transferability of the BG1Luc ER TA was demonstrated by the intra- and inter-laboratory reproducibility studies in the validation study (see **Section 6.0**).

10.1.1 Facilities and Major Equipment

The facility requirements necessary to conduct the BG1Luc ER TA include a standard laboratory setup for sterile cell culture procedures. The major equipment necessary is readily available and includes a laminar flow hood, cell culture incubator, and microplate injecting luminometer. Example suppliers and estimated costs of this equipment are summarized in **Table 10-1**.

Table 10-1 Suppliers and Costs of Major Equipment for the BG1Luc ER TA

Equipment	Example Supplier	Estimated Costs[1]
Laminar Flow Hood	Cole Parmer	$8,000-$12,000
Cell Culture Incubator	Thomas Scientific	$8,000-$15,000
Microplate Injecting Luminometer	Berthold Orion	$28,000

[1]Estimated costs based on 2009 catalog prices

10.1.2 General Availability of Other Necessary Equipment and Supplies

The remaining equipment and supplies necessary to conduct the BG1Luc ER TA (e.g., microscopes, micropipettors, refrigerators/freezers, microtiter plates, cell culture supplies, sera, and reagents) are

35 readily available in most cell culture laboratories or can be readily obtained from any of several scientific
36 laboratory equipment and supply vendors.

37 10.1.3 BG-1Luc4E2 Cell Line

38 The required BG-1Luc4E2 cell line is available upon request from Dr. Michael S. Denison, Department
39 of Environmental Toxicology, University of California, Davis.

40 10.2 BG1Luc ER TA Training Considerations

41 The level of training and expertise needed to conduct the BG1Luc ER TA should be similar to the HeLa-
42 9903 ER TA, the only ER TA currently included on the EPA EDSP Tier 1 screening battery (see **Section**
43 **1.0**). Both methods require a moderate degree of technical capability and a high degree of skill in
44 monitoring and maintaining appropriate cell growth conditions, troubleshooting cell culture problems,
45 and analyzing and interpreting *in vitro* data. Accordingly, personnel should be trained in good cell culture
46 practices, in the specialized culture procedures needed for this assay, and in safety and handling practices
47 appropriate to the types of substances that may be tested in the laboratory (Hartung et al. 2002).

48 It is essential that all laboratory staff be trained to have an awareness of the need to minimize exposure of
49 BG1Luc ER TA to sources of estrogenic contamination, which result in false positive outcomes.

50 10.3 Time and Cost Considerations

51 Most of the necessary equipment for conducting the BG1Luc ER TA is commonly found in laboratories
52 that perform cell culture experiments. The one piece of non-standard laboratory equipment is a microplate
53 injecting luminometer (estimated cost is $26,000), which is required for generating the RLU data used for
54 establishing a positive or negative result in the BG1Luc ER TA.

55 Supplies such as cell culture media, the reagents used to measure luciferase, and cell culture plasticware
56 are available from numerous suppliers. An estimated cost for the BG1Luc ER TA including relevant
57 consumables (cell culture media, reagents, and supplies) is $2000 per test substance for both agonist and
58 antagonist testing (G. Clark, XDS, Inc., personal communication). The BG1Luc ER TA takes
59 approximately two days to perform (this includes a range finder test and at least one comprehensive test).
60 The time estimate for BG1Luc ER TA is similar to the two days necessary to conduct the CERI STTA.
61 The current cost of the CERI STTA assay conducted at CERI ranges from a minimum of $1800 per test
62 article based on at least 11 substances tested to a maximum of $2500 per test article when one to five
63 substances are tested (A. Ono, CERI, personal communication).

64 Commercially available *in vivo* test methods that are used to evaluate estrogenic activity are the
65 Uterotrophic and Female Pubertal assays, which take approximately 30 and 60 days to perform

respectively. The current approximate costs of commercially available Uterotrophic and Female Pubertal assays per test substance are $40,000 and $140,000 respectively (Willet and Sullican 2010).

Hartung T, Balls M, Bardouille C, Blanck O, Coecke S, Gstraunthaler G, et al. 2002. Good cell culture practice: ECVAM good cell culture practice task force report 1. Altern Lab Anim 30(4): 407-414.

ICCVAM. 2003. ICCVAM Evaluation of In Vitro Test Methods For Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays.National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/endo_docs/edfinalrpt0503/edfinrpt.pdf

Willet C, Sullican KM. 2010. Application of an Intelligent Testing Strategy to the US EPA Endocrine Disruptor Screening Program. In: 7th World Congress on Animal Alternatives. Berlin, Germany.