

# Stably Transfected Human Estrogen Receptor-Transactivation Assay for Detection of Estrogenic Agonist and Antagonist Activity of Chemicals

*Commonly used acronym: ER? CALUX assay, ER TA assay, OECD TG 455*

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## Organisation

**Name of the organisation** Vlaamse Instelling voor Technologisch Onderzoek (VITO)

**Department** Health

**Country** Belgium

**Geographical Area** Flemish Region

## SCOPE OF THE METHOD

<b>The Method relates to</b>	Animal health, Environment, Human health
<b>The Method is situated in</b>	Regulatory use - Routine production, Translational - Applied Research
<b>Type of method</b>	In vitro - Ex vivo
<b>Specify the type of cells/tissues/organs</b>	U2OS cell line, originating from Bone Osteosarcoma Epithelial Cells

## DESCRIPTION

### Method keywords

Human U2OS cell line

Transactivation assay

Receptor-ligand complex

hER -mediated transactivation

Reporter genes  
Luciferase reporter plasmids  
Human estrogen receptor alpha  
Luciferase enzyme  
cytotoxicity

### **Scientific area keywords**

Hormone-mediated response  
Endocrine disruption  
Estrogenic activity  
Anti-estrogenic activity  
Chemical testing  
hazard assessment

### **Method description**

The ER CALUX transactivation assay is described in Annex 4 of the OECD TG 455. It uses the human U2OS cell line to detect estrogenic agonist and antagonist activity mediated through human estrogen receptor alpha (hER). This test method is specifically designed to detect hER-mediated transactivation by measuring bioluminescence as the endpoint. The bioassay is used to assess ER ligand binding and subsequent translocation of the receptor-ligand complex to the nucleus. In the nucleus, the receptor-ligand complex binds specific DNA response elements and transactivates a firefly luciferase reporter gene, resulting in increased cellular expression of the luciferase enzyme. Following the addition of the luciferase substrate luciferine, the luciferine is transformed into a bioluminescent product. The light produced can easily be detected and quantified using a luminometer.

### **Lab equipment**

Luminometer for multi-well plates

### **Method status**

Validated by an external party (e.g. OECD, EURL ECVAM,...)

## **PROS, CONS & FUTURE POTENTIAL**

## Advantages

This method can be used for screening and prioritization purposes, but also to provide mechanistic information that can be used in a Weight of Evidence approach to assess endocrine properties of chemicals or mixtures.

## Challenges

- Concentrations of phytoestrogens or other similar compounds higher than 1  $\mu\text{M}$  can over-activate the luciferase expression.
- The test addresses transactivation induced by chemical binding to the ERs in an *in vitro* system. Thus, results should not be directly extrapolated to the complex signaling and regulation of the intact endocrine system *in vivo*.
- Only mycoplasma free cell cultures should be used: cell batches used should either be certified negative for mycoplasma contamination, or a mycoplasma test should be performed before use. RT-PCR should be used for sensitive detection of mycoplasma infection.

For more information see OECD TG 455

## Modifications

Only single substances were used during the validation, the applicability to test mixtures has not been addressed. The test method is theoretically applicable to the testing of multi-constituent substances and mixtures. Before using it on a mixture for generating data for an intended regulatory purpose, it should be considered whether, and if so why, it may provide adequate results for that purpose. The interference of potential cytotoxicity should always be considered.

For more information see OECD TG 455

## REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

### References

Van der Burg B, Winter R, Weimer M, Berckmans P, Suzuki G, Gijsbers L, Jonas A, van der Linden S, Witters H, Aarts J, Legler J, Kopp-Schneider A, Bremer S. (2010). Optimization and prevalidation of the *in vitro* ERalpha CALUX method to test estrogenic and antiestrogenic activity of compounds. *Reprod Toxicol.* 30 (1), 73-80.

## Associated documents

[OECD Test No. 455.pdf](#)

## Links

[Annex 4 of OECD TG 455: Stably Transfected Human Estrogen Receptor- Transactiv...](#)  
[Van der Burg et al. 2010](#)

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