Assessment of estrogenic or anti-estrogenic activity of chemicals by the human stably transfected estrogen sensitive MELN cell line

Commonly used acronym: hER assay with MELN cell line

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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

<table>
<thead>
<tr>
<th>The Method relates to</th>
<th>Animal health, Environment, Human health</th>
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</thead>
<tbody>
<tr>
<td>The Method is situated in</td>
<td>Translational - Applied Research</td>
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<tr>
<td>Type of method</td>
<td>In vitro - Ex vivo</td>
</tr>
<tr>
<td>Species from which cells/tissues/organs are derived</td>
<td>Human</td>
</tr>
<tr>
<td>Type of cells/tissues/organs</td>
<td>Breast cancer cells (transfected)</td>
</tr>
</tbody>
</table>

DESCRIPTION

Method keywords

hER transactivation assay
MELN cell line
cytotoxicity assessment
luciferase activity

Scientific area keywords
endocrine disruption of chemicals
estrogen activity

Method description
MELN cells [provided by INSERM, Montpellier, FR; Balaguer et al. (1999)] are oestrogen-sensitive human breast cancer cells (MCF-7) stably transfected with the oestrogen-responsive gene (ERE- Glo-Luc-SVNeo) carried by integrated plasmids. A standard set-up has been developed to expose MELN cells and measure ER-transactivation for xeno-oestrogenic compounds. Cells are exposed for 19-20 hours to dilutions of test compounds (dissolved at 0.1% DMSO) in comparison to a dilution series of the positive control, 17b-estradiol. At the end of the incubation period, the remaining medium is removed for analysis of cell damage using the CytoTox-ONE Homogenous Membrane Integrity Assay (Promega) as described by Berckmans et al. (2007). Next, cells are lysed and luciferase assay is applied. The hER activation is expressed as percentage of luciferase induction by the vehicle control, and concentration response curves are generated (Graphpad Prism software). The estrogenic potency of chemicals is assessed through comparison of EC50 values relative to the EC50 of 17b-estradiol.

Lab equipment
Laminar flow;
Cell incubator;
Microplate reader for fluorescence and luminescence detection.

Method status
History of use
Internally validated
Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages
High-throughput method for screening of estrogen activity; Agonist or antagonist mode.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References


Associated documents

Witters et al Repr Tox 30-2010, 60-72.pdf