“Next generation mechanistic genotoxicity testing”

The ToxTracker assay

Highly sensitive and specific stem cell-based assay for genotoxicity screening
A unique combination of reporter genes in mouse embryonic stem cells allows accurately identification of genotoxic carcinogens.

Fast, easy and reliable assay for high throughput testing
Easy detection of GFP reporter induction by flow cytometry allows identification of genotoxic properties within 2 days.

Unique genotoxicity assay that provides insight in the biological reactivity of chemicals
The combination of different reporters indicate the cellular signaling pathways that are activated upon exposure to compounds.

Quantitative discrimination between the major initiators of cytotoxicity in a single assay
Combination of different reporters discriminates between direct DNA damage, oxidative stress and protein damage as primary toxic response.

### TOXTRACKER PRINCIPLE

- **Toxicogenomics**
- **Signaling pathways**
- **Pathway-specific mES reporter cells**

### THE TOXTRACKER REPORTERS

<table>
<thead>
<tr>
<th>Biological damage</th>
<th>Cellular pathway</th>
<th>Biomarker</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA damage</td>
<td>ATR/Chk1 DNA damage signaling</td>
<td>Bsci2</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>Nrf2 antioxidant response</td>
<td>Srxn1</td>
</tr>
<tr>
<td>Protein damage</td>
<td>Unfolded protein response</td>
<td>Ddit3</td>
</tr>
<tr>
<td>Cellular stress</td>
<td>p53 signaling</td>
<td>Btg2</td>
</tr>
</tbody>
</table>
TECHNICAL INFORMATION

ToxTracker is based on mouse embryonic stem (mES) cells

- Untransformed, non-cancerous mammalian cells.
- Infinite lifespan.
- Proficient in all major DNA damage signaling and cell cycle regulation pathways.

GFP reporters created using BAC technology

![BAC technology diagram]

**PROTOCOL**

- The ToxTracker assay is performed in 96-wells cell culture plates.
- 24 h. exposure to 5 serial dilutions, typically 2-fold.
- Inclusion of a metabolising system using aroclor-1254 induced rat liver S9
- Detection GFP reporter expression by flow cytometry.

**TECHNICAL INFORMATION**

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GFP reporters created using BAC technology

BAC eGFPires Neo

Gene
- RecE
- RecT
- Physiological promoter
- Homologous recombination

**VALIDATION OF THE TOXTRACKER ASSAY**

Genotoxic carcinogens
- ECVAM class I
- Additional genotoxins (ToxCast)
- Non-genotoxic carcinogens / non-carcinogens
  - ECVAM class II
  - Non-genotoxic carcinogens
  - Non-carcinogens (ToxCast)

Non-genotoxic carcinogens
- ECVAM class I
- Additional genotoxins (ToxCast)
- Non-genotoxic carcinogens
- Non-carcinogens (ToxCast)
- Ambiguous carcinogens (ROS, UPR)
  - ECVAM class III
  - Oxidative stress-inducing agents
  - Protein damage-inducing agents

* Summary of 94 tested compounds
* Percentages indicate the number of compounds that were correctly classified according to their known biological reactivity.

- >95% accuracy

**SPECIFICITY OF THE TOXTRACKER ASSAY**

- DNA damage
- Oxidative stress
- Protein damage

**DATA ANALYSIS**

- custom-made software (Windows, macOS, Linux).
- Automated data integration for GFP reporter activation and toxicity.
- Statistical analysis, data clustering and heatmap creation.

**CONTACT**

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