

## Reporter Cell Lines for Drug Target Identification and Drug Safety Screening

All cellular responses – both beneficial and toxic – are controlled by a complex network of signalling cascades. Effects of chemicals on these pathways can be measured in cells by developing reporter systems, where the promoter of a gene activated by one of these pathways is linked to a reporter.

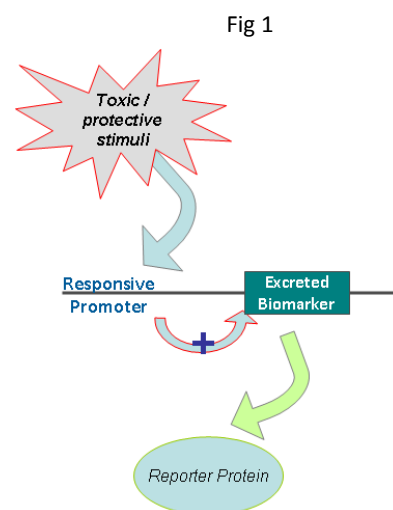
CXR has developed a panel of novel *in vitro* reporter systems using an excreted biomarker (Fig 1). This panel can detect responses such as:

- Oxidative stress & anti-oxidant response
- Inflammatory response
- Cell cycle control, DNA damage & apoptosis
- Hypoxia/angiogenesis
- Toxic & other stress responses

The reporter cell lines can therefore be used to generate mechanistic information on the properties of a compound or series of compounds. In particular, the cell lines can be used to:

- Screen anti-cancer / cytotoxic / cytoprotective compound libraries – giving a rapid & informative assessment of mechanisms of action
- Screen drug candidates for potential mechanisms of toxicity in a cost-effective high throughput format

The easily-measured excreted biomarker is attached to a gene promoter region in such a way that, when there is a change in expression of that particular gene, this is reflected by an increase or decrease in levels of the marker. The biomarker can be detected in the culture medium using commercially-available ELISA assays. Ten cell lines containing promoters associated with oncogenic / cytoprotective / toxic response pathways have been developed.



### Reporter Cell Line Advantages

<i>Easy &amp; simple measurement</i>	<ul style="list-style-type: none"> <li>• Directly from cell culture medium – no preparation of sample needed</li> <li>• Simple ELISA assay (widely available commercial kits)</li> </ul>
<i>Accuracy</i>	<ul style="list-style-type: none"> <li>• Little to no constitutive expression of biomarker, high induced expression</li> <li>• Production/induction of biomarker reflects test item effects</li> </ul>
<i>Multiple mechanistic models available</i>	<ul style="list-style-type: none"> <li>• 10 validated reporter lines currently available to enable the identification of mechanisms of action and toxicity</li> <li>• Complementary screens possible. For example, using the ARE line to screen for induction of antioxidant pathways and the p21 line to screen for DNA damage gives mechanistic information as to whether your compound is a protective anti-oxidant or an electrophile</li> </ul>
<i>Time-dependent response</i>	<ul style="list-style-type: none"> <li>• Flexible measurement system enabling multiple timepoint sampling</li> <li>• Reflects time-dependent effects of test item</li> </ul>
<i>Flexibility to measure Up or Down-regulation</i>	<ul style="list-style-type: none"> <li>• Up-regulation – simple incubation with test item</li> <li>• Down-regulation – co-incubation with known inducers of stress response</li> </ul>
<i>Reactive metabolite detection</i>	<ul style="list-style-type: none"> <li>• Assay cell lines can be transfected with a choice of human cytochrome P450s to evaluate metabolites</li> <li>• Comparison with control, P450 free cells can demonstrate reactive metabolite effect</li> </ul>

## Available Reporter Cell Lines

Biological Process	Promoter/Enhancer	Cell Line	
<i>Oxidative Stress, Antioxidant Response</i>	<i>Antioxidant Response Element (ARE)</i>	MCF7/ ARE, 3-66	<ul style="list-style-type: none"> <li>Cellular response to electrophilic compounds, xenobiotics and antioxidants</li> </ul>
	<i>Hmox1</i>	CHO/ Hmox1, 3-94	<ul style="list-style-type: none"> <li>Cellular response to oxidative stress</li> </ul>
<i>Inflammation &amp; Immunity</i>	<i>NFkB</i>	HeLa/ NFkB, 57	<ul style="list-style-type: none"> <li>Immune responses, inflammation, cell growth, apoptosis, tumorigenesis</li> </ul>
<i>Cell Cycle Control, DNA Damage, Apoptosis</i>	<i>TPA Response Element (AP-1)</i>	HeLa/ AP-1, 53	<ul style="list-style-type: none"> <li>Proliferation, survival, differentiation and transformation</li> </ul>
	<i>p53</i>	A2780/ p53	<ul style="list-style-type: none"> <li>Cell cycle control, DNA damage and apoptosis</li> </ul>
	<i>Cyclin-dependent kinase inhibitor-1A (p21<sup>waf-1</sup>)</i>	A2780/ p21 <sup>waf1</sup>	<ul style="list-style-type: none"> <li>DNA damage, cell cycle control</li> </ul>
<i>Angiogenesis, Hypoxia</i>	<i>Hypoxia Response Element</i>	MCF7/ HRE, 2-51	<ul style="list-style-type: none"> <li>Angiogenesis, glucose metabolism, cell proliferation/survival and invasion/metastasis</li> </ul>
<i>Toxic and other stress responses</i>	<i>Xenobiotic Response Element</i>	MCF7/ XRE, 2-9	<ul style="list-style-type: none"> <li>Response to particular classes of toxic and carcinogenic compounds</li> </ul>
	<i>Heat Shock Protein 70</i>	HeLa/ HSP70	<ul style="list-style-type: none"> <li>General cellular response to stress, toxic response to e.g. heavy metals</li> </ul>
<i>Cell Growth &amp; Differentiation</i>	<i>Glucocorticoid Response Element</i>	HeLa/ GRER, 44	<ul style="list-style-type: none"> <li>Cell growth &amp; differentiation</li> </ul>

Considerable technical expertise and experimental know-how has identified the optimal:

- Cell line for each promoter
- Promoter sequence
- Promoter repeat number

The above lines cover the major pathways of cytoprotection and cellular stress, and have been created as a result of collaborations with Millipore Inc. The cell lines are freely available for licensing or fee-for-service work.