

AREc32 cell line

An *in vitro* screen for electrophilic and antioxidant properties of chemicals



An *in vitro* screen for electrophilic and antioxidant properties of chemicals

Schematic representation of the Nrf2/ARE pathway. This pathway can be activated by cytoprotective agents or in response to electrophiles. Ultimately Nrf2 will cause activation of the cytoprotective genes under ARE regulation.



The AREc32 cell line: A reproducible and reliable *in vitro* assay for activation of the Antioxidant Response Element

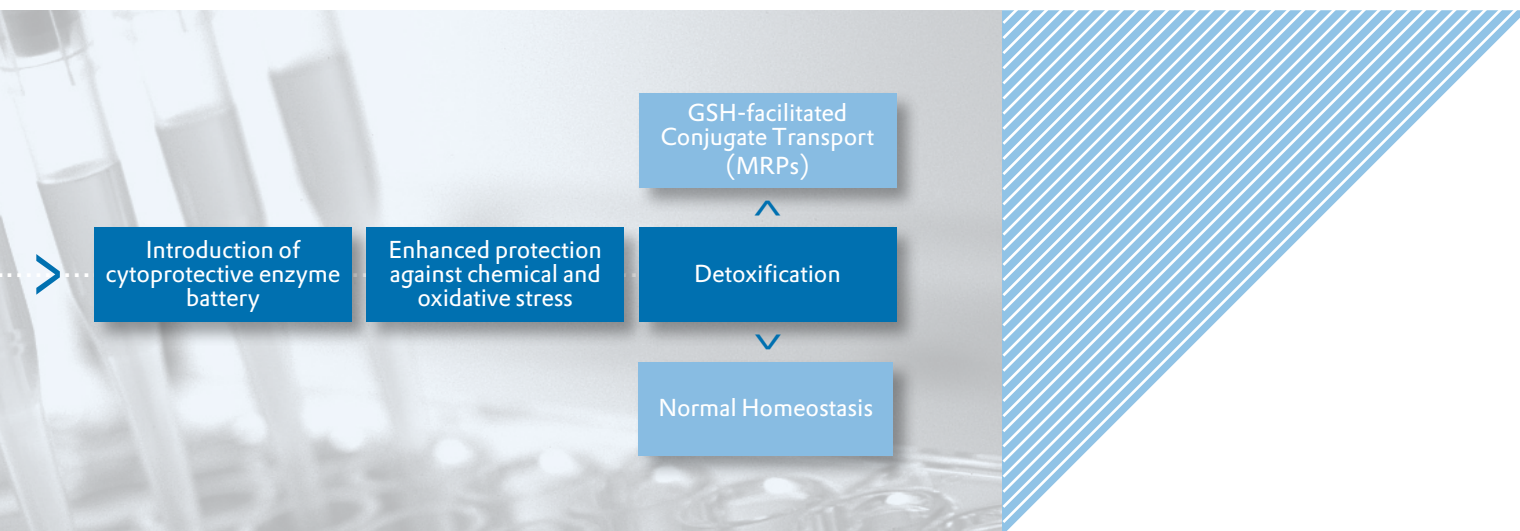
The AREc32 stable cell line (Ref1) can be used in a screen for both cytoprotective and electrophilic compounds. It is a reproducible and reliable *in vitro* assay that measures Nrf2 mediated activation of the Antioxidant Response Element (indicative of cytoprotective enzyme induction) via a luciferase reporter transgene.

The cell line has been validated with many compounds that are known ARE activators. For example, tert-butylhydroquinone (tBHQ), a phenolic antioxidant and known inducer of enzymes that protect against oxidative stress, resulted in induced luciferase activity up to 70-fold higher than basal levels, enabling an accurate read-out of ARE activation.

Background

The antioxidant response element (ARE) is a transcriptional regulatory element involved in the activation of genes coding for a number of antioxidant proteins and detoxifying enzymes. These enzymes work in concert to protect tissues from oxidative insults and chemical toxicities e.g. aldo keto reductase, NAD(P)H: quinone oxidoreductase1 and glutathione-S-transferases.

The key protein that links many chemicals to the ARE is the transcription factor Nrf2 (NF-E2-related factor). Nrf2 levels are constitutively low as a consequence of its interaction with Keap1, which targets its degradation. Chemicals can react with key cysteine residues in Keap1, resulting in Nrf2 accumulation in the nucleus. Once within the nucleus, Nrf2, in complex with other coactivators such as p300, can bind to the ARE to induce gene transcription of cytoprotective enzymes resulting in the prevention of toxicity.



Assessment and screening system for potential skin sensitisation

Features

The AREc32 cell line is a stably transfected MCF7 cell line that contains a luciferase gene construct under the control of the ARE (eight copies of the rat GSTA2 ARE cis-element – see Ref1). The luciferase reporter provides a rapid and convenient quantification of ARE induction.

Utilities

The same output is generated by an electrophile and an antioxidant, therefore the cell line can be used as both:

- ▶ An efficacy screen to identify compounds with chemoprotective properties.
- ▶ A screen for compounds that induce skin sensitisation.

Benefits

Benefits to using the AREc32 cell line include:

A potential *in vitro* alternative to the LLNA

Screening of compounds to predict activation of cytoprotective antioxidant proteins and pathways, which are perceived to be beneficial to human health

Transient transfection of the AREc32 cell line with human cytochrome P450 genes allows investigation of the effect of metabolites on this pathway

Example

Assessment and screening system for skin sensitisation:

The AREc32 cell line has been compared to the mouse Local Lymph Node Assay (LLNA), (see below and Ref 2). The authors hypothesised that skin sensitisers act via protein/peptide reactivity, such as the covalent modification of cysteine residues that cause activation of Keap1. This study demonstrated the value of the AREc32 assay as an *in vitro* screen for skin sensitisers compared to the LLNA.

Developing an *in vitro* alternative for the LLNA would be beneficial in reducing animal and radioactivity use.

- ▶ Natsch & Emter (Ref 2) tested the AREc32 cell line with 102 chemicals, including 70 known sensitisers

Accuracy was 83% vs. LLNA, compared with 86% for the LLNA vs. guinea pigs and 72% vs. human (NIH, Feb 1999)

14 of 15 strong/extreme sensitisers gave a +ve result, 31 of 35 moderate sensitisers, and 12 of 20 weak sensitisers

Four of 30 non-sensitiser gave a false +ve

Positive predictivity was 93.4%

Negative predictivity was 66.6%

- ▶ The AREc32 cell line could be included in a battery of *in vitro* tests used to examine skin sensitisation (Ref 3).

Founded in 2001, CXR Biosciences has used its collaborative approach, proprietary models and cutting edge expertise to help customers of all sizes solve issues relating to the selection of drug candidates or safety of compounds. CXR offers tailored preclinical services in the areas of drug development and investigative toxicology, as well as developing preclinical technology platforms that are more predictive of drug response in man. CXR's customers include pharmaceutical, chemical & biotechnology companies, leading universities and research institutions. CXR Biosciences Ltd. is located in Dundee, Scotland.

For further information on this or any of our services or technologies, please contact:

CXR Biosciences
2 James Lindsay Place
Dundee DD1 5JJ
Scotland UK

Tel: +44 1382 432163
Fax: +44 1382 432153
Email: info@cxrbiosciences.com
www.cxrbiosciences.com



Access to the AREc32 line

Non-exclusive licences are available for purchase where the cell line will be shipped to the client for in-house use at own site.

Alternatively, assessment of compounds by the AREc32 cell line is offered by CXR Biosciences on a fee-for-service basis (either as a standalone study or part of a wider program).

If more information relating to the AREc32 cell line is required, please contact us by calling 01382 432163 or email: info@cxrbiosciences.com

References

1. Generation of a stable antioxidant response element-driven reporter gene cell line and its use to show redox-dependent activation of Nrf2 by cancer chemotherapeutic agents (2006) Wang, X.J., Hayes, J.D. and Wolf, C.R. *Cancer Res.* 66:10983-94
2. Skin sensitisers induce antioxidant response element dependent genes: Application to the *in vitro* testing of the sensitisation potential of chemicals (2008) Natsch, A. and Emter, R. *Toxicol. Sci.* 102:110-9
3. Filling the Concept with Data: Integrating Data from Different *in vitro* and *in silico* Assays on Skin Sensitizers to Explore the Battery Approach for Animal-Free Skin Sensitisation Testing (2009) Natsch, A., Emter, R. and Ellis, G. *Toxicol. Sci.* 107:106-121

