



**The AREc32 cell line:**  
An *in vitro* screen for electrophilic and  
antioxidant properties of chemicals

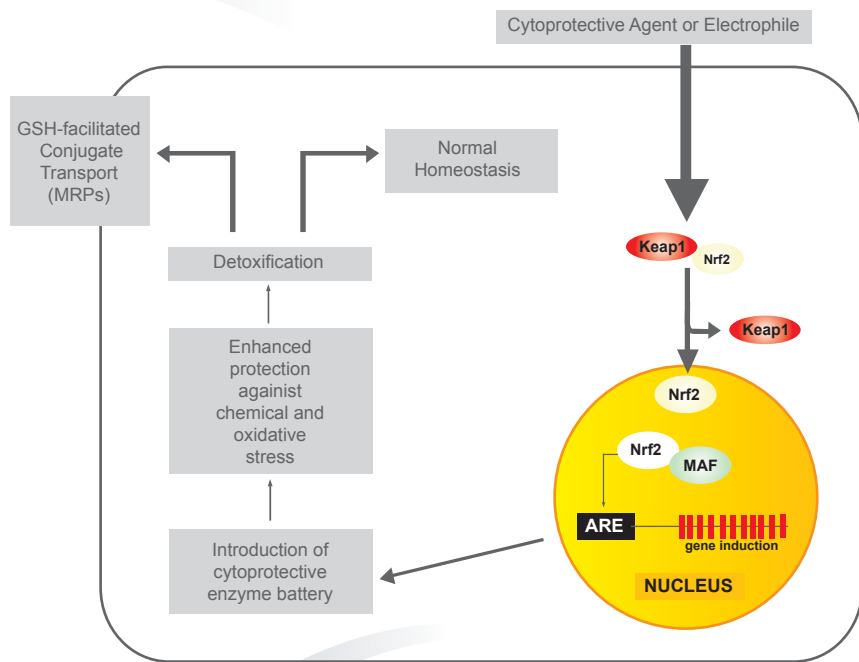
The AREc32 stable cell line (Reference 1) can be used in a screen for both cytoprotective and electrophilic compounds. It is a reproducible and reliable *in vitro* assay that measures Nrf2 mediated activation of the Antioxidant Response Element (indicative of cytoprotective enzyme induction) via a luciferase 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 and include proteins such as aldo keto reductase, NAD(P)H: quinone oxidoreductase 1 and glutathione-S-transferases.

The key common protein that links many oxidative chemicals such as phenolic antioxidants and electrophilic compounds 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. Electrophiles react with key cysteine residues in Keap1, releasing Nrf2 and allowing nuclear translocation. 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 (See Figure 1).

**Figure 1:** Schematic representation of the Nrf2/ARE pathway. This pathway can be activated by cytoprotective agents and additionally in response to electrophiles. Ultimately Nrf2 will cause activation of the cytoprotective genes under ARE regulation.



**Figure 1:** Schematic representation of the Nrf2/ARE pathway.

## Example: Assessment and screening system for skin sensitisation

As a demonstration of its screening potential, the AREc32 cell line has been utilised in a sensitising agent study vs. data gathered with the mouse Local Lymph Node Assay (LLNA), the approved method for identifying substances with immunotoxic properties (see below and Reference 2). The authors hypothesised that skin sensitizers 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 sensitizers compared to the LLNA. Developing an *in vitro* alternative for the LLNA would be beneficial in reducing animal and radioactivity use and will be mandatory from March 2013.

- Natsch & Emter (Reference 2) tested the AREc32 cell line with 102 chemicals, including 70 known sensitizers
  - 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 sensitizers gave a +ve result, 31 of 35 moderate sensitizers, and 12 of 20 weak sensitizers
  - Four of 30 non-sensitizers gave a false +ve
  - Positive predictivity was 93.4%
  - Negative predictivity was 66.6%

## Reactive Metabolites

While the direct effect of administered compounds is important, in many cases the observed toxic or protective effects are due to metabolites. Therefore CXR are developing a number of adenoviral vectors that will allow the expression of single or multiple human cytochrome P450's within *in vitro* systems such as the AREc32 cell line for the investigation of metabolites.

## 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 Reference 1). The luciferase reporter provides a rapid and convenient quantification of ARE induction.

## 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.
- 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 sensitization

Certain electrophiles are also known to damage DNA. Therefore, compounds screened through the AREc32 cell based assay can be further classified as toxins if they activate an *in vitro* p21 DNA damage screen or if they are reactive in a peptide binding assay. Conversely, an antioxidant will fail to activate the p21 screen. All three assays are available at CXR Biosciences.

## Access to the AREc32 line

Non-exclusive licences are available for purchase where the cell line will be shipped to the client for their own in-house assessment of compound induction of the ARE pathway.

Alternatively, assessment of compounds via 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 any other information relating to the AREc32 cell line is required, please contact Audrey Vardy by calling 01382 432163 or email [audreyvardy@cxrbiosciences.com](mailto:audreyvardy@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 sensitizers induce antioxidant response element dependent genes: Application to the *in vitro* testing of the sensitization potential of chemicals (2008) Natsch, A. and Emter, R. *Toxicol. Sci.* 102:110-9

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- 1 **Drug Development Solutions** supporting *informed* decision making, avoiding potential problems and accelerating development.
- 2 **Investigative and Mechanistic Toxicology** by understanding the pathways that define the sensitivity of cells to chemicals, we evaluate the *actual* hazard to man of drugs and chemicals.

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