YOUR WORK: CITATIONS OF RECENT PEER-REVIEWED LITERATURE

REVIEWS BY TERRI SUNDQUIST, PROMEGA CORPORATION

Determining Subcellular Localization of XAF1 Using the HaloTag® Interchangeable Labeling Technology

Straszewski-Chavez, S.L., Visintin, I.P., Karassina, N., Los, G., Liston, P., Halaban, R., Fadiel, A. and Mor, G. (2007) XAF1 mediates tumor necrosis factor-α-induced apoptosis and X-linked inhibitor of apoptosis cleavage by acting through the mitochondrial pathway. *J. Biol. Chem.* **282**, 13059–72.

The authors shed light on the mechanism by which first-trimester trophoblasts resist FAS ligand-induced apoptosis but remain sensitive to TNFα-mediated apoptosis. In response to TNFα treatment, first trimester trophoblasts express XAF1 [X-linked inhibitor of apoptosis (XIAP)-associated factor 1], which antagonizes the caspase inhibitor XIAP. The authors used the HaloTag® pHT2 Vector[a–c], which encodes the 33kDa monomeric HaloTag® reporter protein, to create HaloTag®-XAF1 fusion constructs and transiently transfected the first-trimester trophoblast 3A cell line. Live 3A cells were labeled with the HaloTag® TMR Ligand[a,b], and the HaloTag®-XAF1 fusion protein was shown to localize to the cytoplasm by confocal microscopy. To further characterize the subcellular localization, 3A cells transiently transfected with the fusion construct were separated into cytoplasmic and mitochondrial fractions, the fractions were labeled with HaloTag® TMR Ligand, proteins in each fraction were separated by SDS polyacrylamide gel electrophoresis, and the amount of labeled XAF1 was quantified using a fluorescence imager. XAF1 was found predominantly in the mitochondrial fraction. TNFα treatment of 3A cells induced translocation of endogenous XAF1 to the mitochondria. The authors used the Caspase-Glo® Assays[d–f] to demonstrate activation of caspase-3 and caspase-9 in response to expression of XAF-1. They also showed that caspase-3 activation and XIAP cleavage correlated with translocation of endogenous XAF1 to mitochondria. Viability of 3A and primary trophoblasts overexpressing XAF1 was evaluated using the CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay[g].

Related Products

Product	Size	Cat.#
Caspase-Glo® 3/7 Assay*	2.5 ml	G8090
Caspase-Glo® 9 Assay*	2.5 ml	G8210
CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay*	1,000 assays	G3580
HaloTag® pHT2 Vector	20 µg	G8241
HaloTag® TMR Ligand	30 µl	G8251

*For Laboratory Use.

[a]Patent Pending.

[b]For research use only. With respect to manufacture or sale of research products or services, or any diagnostic, therapeutic or prophylactic uses, please contact Promega for supply and licensing information.

[c]The CMV promoter and its use are covered under U.S. Pat. Nos. 5,168,062 and 5,385,839 owned by the University of Iowa Research Foundation, Iowa City, Iowa, and licensed FOR RESEARCH USE ONLY. Research Use includes contract research for which monetary or other consideration may be received. Other commercial users must obtain a license to these patents directly from the University of Iowa Research Foundation.

[d]U.S. Pat. No. 7,148,030 and other patents pending.

[e]U.S. Pat. Nos. 6,602,677 and 7,241,584, Australian Pat. Nos. 754312 and 785294 and other patents and patents pending.

[f]The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673

[g]The MTS tetrazolium compound is the subject of U.S. Pat. No. 5,185,450 assigned to the University of South Florida and is licensed exclusively to Promega Corporation.

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Characterizing Silybin-Mediated Inhibition of Cytochrome P450 Enzymes and Determining the Cytochrome P450 Isoform Responsible for Silybin Metabolism

Jancová, P., Anzenbacherová, E., Papousková, B., Lemr, K., Luzná, P., Veinlichová, A., Anzenbacher, P. and Simánek, V. (2007) Silybin is metabolized by cytochrome P450 2C8 in vitro. *Drug Metab. Dispos.* **35**, 2035–9.

Silybin is a flavonolignan that acts as a hepatoprotectant, has anticancer, chemoprotective and neuroprotective activities and inhibits cytochrome P450 (P450) enzyme activity. The authors quantified silybin-mediated inhibition of P450s in human liver microsomal fractions and characterized the role of individual human liver P450 enzymes in the metabolism of silybin. Silybin did not or only weakly inhibited CYP2E1, 2A6, 2B6, 2C19 and 2D6 ($IC_{50} \geq 250$ µM), moderately inhibited CYP1A2 and 2C8, and had the greatest effect on CYP3A4 and 2C9 ($IC_{50} \leq 50$ µM). CYP2C8 activity was measured using the P450-Glo™ CYP2C8 Assay[a,b]. To determine which P450 isoforms were responsible for silybin metabolism, specific P450 inhibitors were added to P450 reactions, and *O*-demethylated silybin, the main silybin metabolite, and hydroxylated derivatives of silybin were quantitated by microspray liquid chromatography-mass spectrometry. These studies as well as experiments performed using *E. coli* membrane fractions containing recombinant human P450 enzymes confirmed that CYP2C8 is responsible for the formation of the *O*-demethylated derivative.

Related Products

Product	Size	Cat.#
P450-Glo™ CYP2C8 Assay	10 ml	V8781
	50 ml	V8782

[a]U.S. Pat. Nos. 6,602,677 and 7,241,584, Australian Pat. Nos. 754312 and 785294 and other patents and patents pending.

[b]The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.

p450-Glo is a trademark of Promega Corporation.

YOUR WORK: CITATIONS OF RECENT PEER-REVIEWED LITERATURE

REVIEW BY TERRI SUNDQUIST, PROMEGA CORPORATION

Developing a High-Throughput β-Galactosidase Assay Using a Luminogenic Substrate For Use in *Saccharomyces cerevisiae*

de Almeida, R.A., Burgess, D., Shema, R., Motlekar, N., Napper, A.D., Diamond, S.L. and Pavitt, G.D. (2008) A *Saccharomyces cerevisiae* cell-based quantitative β-galactosidase assay compatible with robotic handling and high-throughput screening. *Yeast* **25**, 71–6.

β-galactosidase (β-gal) is a common reporter protein in yeast, but β-gal assays are not ideally suited for high-throughput screening (1). In this study, the authors adapted the Beta-Glo® Assay System[a–c], which uses a luciferin-galactoside substrate in a coupled-enzyme assay with firefly luciferase to generate light, for use as a high-throughput reporter assay in yeast. The modifications to the Beta-Glo® assay protocol included addition of a protease inhibitor-EDTA cocktail to the yeast cell cultures to try to enhance and stabilize light ouput and dilution of the cultures in an SDS-containing buffer to improve cell lysis. The resulting assay was reproducible; simple to perform, with minimal sample handling and no centrifugation steps; and sensitive, detecting β-gal activity in <100 cells. Light output was stable for >8 hours and proportional to the number of yeast cells over at least three orders of magnitude. In addition, the assay was compatible with yeast expressing β-gal from a plasmid-encoded or integrated copy of the *lacZ* gene and yeast grown in complex or defined media. Using robotic liquid-handling systems, the modified Beta-Glo® Assay was scaled down for use in 96-well and 384-well plates, using only 1–5 µl of cell culture and 10–50 µl of the Beta-Glo® Assay Reagent per assay.

The authors also compared the results of their modified Beta-Glo® Assay with the results of a traditional colorimetric β-gal assay. Assays performed in parallel using the Beta-Glo® Assay Reagent and colorimetric substrate yielded equivalent results, but the Beta-Glo® Assay did not require cell extract preparation, reducing assay time and minimizing variation observed for replicate samples with the β-gal assay.

Reference

1. Bovee, T.F.H *et al.* (2004) Development of a rapid yeast estrogen bioassay, based on the expression of green fluorescent protein. *Gene* **325**, 187–200.

Related Products

Product	Size	Cat.#
Beta-Glo® Assay System	10 ml	E4720
	100 ml	E4740
	10 × 100 ml	E4780

[a]U.S. Pat. Nos. 6,602,677 and 7,241,584, Australian Pat. Nos. 754312 and 785294 and other patents and patents pending.

[b]The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.

[c]Certain applications of this product may require licenses from others.

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