# CITATION NOTE: USING THE APO-ONE® ASSAY IN TISSUE EXTRACTS AND USING LUCIFERASE REPORTERS TO STUDY HYPOXIA-INDUCED GENE EXPRESSION

REVIEW BY MICHELE ARDUENGO, PH.D., PROMEGA CORPORATION

Wagner, K.-D. *et al.* (2003) Oxygen-regulated expression of the Wilms' tumor suppressor *Wt*1 involves hypoxia-inducible factor-1 (HIF-1). *FASEB J.* **17**, 1364–6.

The authors of this study investigate the effects of hypoxia on expression of the Wilms' tumor gene (*Wt*1). In this study, the authors try to determine if hypoxia-inducible factor-1 (HIF-1) regulates an HRE element in the *Wt*1 promoter. They also describe a correlation between hypoxia, *Wt*1 expression and a decrease in apoptosis in renal tubules.

## Inducible Luciferase Activity With the *Wt*I Promoter

To investigate the expression of the *Wt*1 gene in response to hypoxic conditions, the authors used PCR to clone the promoter of the *Wt*1 gene. The promoter was cloned into the pGL2 Basic Vector[a] (Cat.# E1641) and used to drive the luciferase reporter. They showed that the *Wt*1 promoter mediated induction of the luciferase gene in response to hypoxia (1% $O_2$ v. 20% $O_2$). In all experiments, luciferase activity was normalized to a β-galactosidase control[b] using the β-galactosidase Enzyme Assay System (Cat.# E2000). The same response could be obtained by treating the cells with $CoCl_2$, which is believed to deplete $Fe^{2+}$ in cells, a critical cofactor for HIF-1α prolyl hydroxylase. This hydroxylase catalyzes the rate-limiting step in the degradation of HIF-1α (hypoxi-inducible factor-1), and HIF-1α is a candidate protein for regulating the *Wt*1 hypoxia induction.

The authors generated mutations in each of the two HRE sites of the *Wt*1 promoter and repeated the experiment. Mutation of the 5´ HRE site abolished responsiveness of the reporter to HIF-1α expression and $CoCl_2$ treatment.

## Measuring Apoptosis in Kidney Tissue Extracts

Because *Wt*1 is a transcriptional activator of Bcl-2, the authors investigated apoptosis in renal tissues from hypoxic and normoxic rats. One measure of apoptosis is caspase-3/7 activity. Activation of caspases is an early event in the apoptotic pathway. The authors used the Apo-ONE® Homogeneous Caspase-3/7 Assay (Cat.# G7792) with kidney homogenate samples. Rats were subjected to normoxic conditions (20% $O_2$) or hypoxic conditions (8% $O_2$). Kidney tissue from the rats was homogenized in 25mM HEPES (pH 7.5), 0.1% Triton® X-100, 5mM $MgCl_2$, 2mM DTT, 74µM antipain, 0.15µM aprotinin, 1.3mM EDTA, 1mM EGTA, 15µM pepstatin, and 20µM leupeptin. The authors centrifuged the homogenates at 50,000 × *g,* and supernatants were removed and assayed following the protocol in the *Apo-ONE® Homogeneous Caspase-3/7 Assay Technical Bulletin* #TB295.

The authors describe a 37% decrease in caspase-3/7 activity in the homogenates from the hypoxic rats and suggest the possibility that *Wt*1 is upregulating Bcl-2 in these tissues and reducing apoptosis. This hypothesis is further supported by TUNEL assays that indicated 4X fewer TUNEL-positive cells in the hypoxic tissues and an increase in the expression of Bcl-2. ■

## Protocols

*Apo-ONE® Homogeneous Caspase-3/7 Assay Technical Bulletin* #TB295
(www. promega.com/tbs/tb295/tb295.html)

*pGL2 Luciferase Reporter Vectors Technical Manual* #TM003
(www.promega.com/tbs/tm003/tm003.html)

β-*Galactosidase Enzyme Assay System with Reporter Lysis Buffer Technical Bulletin* #TB097
(www.promega.com/tbs/tb097/tb097.html)

## Ordering Information

| Product | Size | Cat.# |
|---|---|---|
| Apo-ONE® Homogeneous Caspase-3/7 Assay | 1ml | G7792 |
| | 10ml | G7790 |
| | 100ml | G7791 |
| β-Galactosidase Enzyme Assay System with Reporter Lysis Buffer[b] | 1 each | E2000 |
| pGL2-Basic Vector[a] | 20µg | E1641 |

[a] 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. A license (from Promega for research reagent products and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.

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

Apo-ONE is a trademark of Promega Corporation and is registered with the U.S. Patent and Trademark Office.

Triton is a registered trademark of Union Carbide Chemicals and Plastics Technology Corporation.