# What can you do with a luciferase Reporter Assay?





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#### **Application Overview**



#### **Traditional Nuclear Receptor Assays**



#### Your expression analysis found an uncharacterized NR...





#### **Universal Nuclear Receptor Assays**

Ligand binding domain responsible for: homodimerization (class I receptors) heterodimerization (class II receptors) corepressor binding coactivator binding **Nuclear Receptor** Ligand Binding Domain pBIND Vector GAL4 BD GAL4 UAS GAL4 UAS GAL4 UAS GAL4 UAS GAL4 GAL4 GAL4 GAL4 TATA luc2P UAS UAS UAS UAS UAS Box

pGL4.35[/uc2P/9XGAL4UAS/Hygro] Vector



## What you need...



#### **Nuclear Receptor Assay Principle**





# Pre-designed tools available for nuclear receptor assays



#### **Case Study: Coactivator of Vitamin D receptor**

Pright C 2005 The American Society for Pharmacology and Experimental Therapeut Pharmacol 68:511-517, 2005 Coactivation of the Human Vitamin D Receptor by the Peroxisome Proliferator-Activated Receptor  $\gamma$  Coactivator-1  $\alpha$ Rajesh S. Savkur, Kelli S. Bramlett, Keith R. Stayrook, Sunil Nagpal, and Thomas P. Burris Ell Lilly and Company, Lilly Research Laboratories, Indianapolis, Indiana (R.S.S., K.S.B., K.R.S., S.N., T.P.B.); and Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, Indiana (K.S.B., T.P.B.) Received March 9, 2005; accepted May 20, 2005 ABSTRACT The vitamin D Receptor (VDR) belongs to the superfamily of steroid/thyroid hormone receptors that is activated by 1a,25serve as a coactivator for VDR. Transient cotransfection assays demonstrate that PGC-1 $\alpha$  augments ligand-dependent VDR dihydroxyvitamin D<sub>3</sub>. Traditional targets for 1a,25-dihydroxyvitranscription when either full-length VDR or Gal4 DNA binding domain-VDR-ligand binding domain chimeras were analyzed tamin D, action include tissues involved in the maintenance of calcium homeostasis and bone development and remodeling. Furthermore, mammalian two-hybrid assays, coimmunopre-Peroxisome proliferator-activated receptor v coactivator-1a cipitation analyses, and biochemical coactivator recruitment PGC-1 $\alpha$ ), a transcriptional coactivator that plays a role in miassays demonstrate a ligand-dependent interaction between tochondrial biogenesis and energy metabolism, is predomi-nantly expressed in kidney, heart, liver, and skeletal muscle. the two proteins both in cells and in vitro. The coactivatio potential of PGC-1α requires an intact AF-2 domain of VDR and Because VDR and PGC-1 $\alpha$  display an overlapping pattern of expression, we investigated the possibility that PGC-1 $\alpha$  could the LXXLL motif in PGC-1a. Taken together, these results in dicate that PGC-1a serves as a coactivator for VDR. The hormonally active form of vitamin D, 1a,25-dihytionally divided into three distinct regions: an N-terminal droxyvitamin D<sub>3</sub> (1,25(OH)<sub>2</sub>D<sub>3</sub>) or calcitriol, and its synthetic region containing a ligand-independent activation func analogs mediate their biological actions via the vitamin D tion-1, a highly conserved central region containing the DNA receptor (VDR; NR1I1), a member of the nuclear receptor binding domain (DBD), and the C-terminal region of the superfamily (van Leeuwen et al., 2001). The actions of receptor containing a multifunctional domain harboring the 1,25(OH)<sub>2</sub>D<sub>3</sub> include maintenance of calcium homeostasis ligand binding domain (LBD), the RXR heterodimerization and bone development and remodeling (DeLuca, 2004). In motif, and a ligand-dependent activation function-2 (AF2) addition, vitamin D<sub>3</sub> has also been shown to function in the (Mangelsdorf et al., 1995; Giguere, 1999; Burris, 2001). The differentiation of leukemic cells (Abe et al., 1981; Bar-Shavit binding of a ligand to the receptor induces a conformational et al., 1983; Mangelsdorf et al., 1984), inhibit the growth and change within the LBD of VDR, resulting in the release of proliferation of various cancerous cells (Colston et al., 1981; bound corepressor proteins and permitting the association of Dokoh et al., 1984; Skowronski et al., 1993), and possess coactivator proteins that mediate a series of events leading to immunosuppressive activity (Manolagas et al., 1985). The transcriptional activation of target genes (Glass and Rosenphysiological and pharmacological actions of these comfeld, 2000; Savkur and Burris, 2004). The traditional target pounds have indicated the application of VDR ligands in tissues exhibiting hormone-dependent VDR actions include inflammation, autoimmune diseases, osteoporosis, cancers, tissues such as the bone, kidney, small intestine, parathyroid and dermatological indications (Pinette et al., 2003). glands, skeletal muscle, heart, skin, breast, colon, prostate, The VDR protein is modular in nature and can be funcand gonads. VDR response elements (VDREs) have been identified in the promoters of vitamin D<sub>3</sub> responsive genes Article, publication date, and citation that are essential for the hormonal responsiveness of the http://molpharm.aspetjournals.org. doi:10.1124/mol.105.012708. tissues including calbindin-D<sub>DK</sub> (Darwish and DeLuca, 1992), ABBREVIATIONS: 1.25(OH)<sub>2</sub>D<sub>3</sub>, 1a,25-dihydroxyvitamin D<sub>3</sub>, OPN, osteopontin; PGC-1a, peroxisome proliferator-activated receptor y coactiva-tor-1a; VDR, vitamin D receptor; DBD, DNA binding domain; LBD, ligand binding domain; AF, activation function; VDRE, vitamin D response element; PCR, polymerase chain reaction; GST, glutathione S-transferase; HEK, human embryonic kidney; DMSO, dimethyl sulfoxide; GFP, greer fluorescent protein; PPAR, peroxisome proliferator-activated receptor; FXR, famesoid X receptor; RXR, 9-cis retinoid X receptor; NR, nuclea eptor; TR, thyroid receptor; ER, estrogen receptor

Vitamin D receptor (VDR) is a nuclear receptor that upon ligand binding displaces a co-repressor and allows co-activators to bind, allowing transcription.

Central question:

- Peroxisome Proliferator-Activated Receptor γ coactivator-1 α (PGC-1α) and the Vitamin D receptor have overlapping tissue distribution,
- Does PGC1α function as a coactivator of VDR?

Savkur, R.S., et al. (2005) Mol. Pharmacol. 68, 511-517.



### **PGC-1** $\alpha$ is a coactivator of VDR



**Case Study:** 

Savkur, R.S. et al.

Conclusions supported by work with full receptor & mammalian two-hybrid work



Fold Increase Luc

activity

1

5

1

up to

500

1

1

#### **More Information...**





Paguio, A., et al. (2008) Improvements to luciferase reporter assays for nuclear receptor function. PS065 presented at *Society for Biomolecular Sciences* meeting.

