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## PathScan® Hydroxy-HIF-1α (Pro564) Sandwich ELISA Kit

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**Species Cross Reactivity:** H  
**UniProt ID:** #Q16665  
**Entrez-Gene ID:** #3091

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**For Research Use Only. Not for Use in Diagnostic Procedures.**

Product Includes	Product #	Quantity	Color	Storage Temp
HIF-1α Goat Antibody Coated Microwells	25917	96 tests		+4C
Hydroxy-HIF-1α (Pro564) Rabbit Detection mAb	14427	1 ea	Green (Lyophilized)	+4C
Anti-rabbit IgG, HRP-linked Antibody (ELISA Formulated)	13272	1 ea	Red (Lyophilized)	+4C
Detection Antibody Diluent	13339	11 ml	Green	+4C
HRP Diluent	13515	11 ml	Red	+4C
TMB Substrate	7004	11 ml	Colorless	+4C
STOP Solution	7002	11 ml	Colorless	+4C
Sealing Tape	54503	2 ea		+4C
ELISA Wash Buffer (20X)	9801	25 ml	Yellowish	+4C
ELISA Sample Diluent	11083	25 ml	Blue	+4C
Cell Lysis Buffer (10X)	9803	15 ml	Yellowish	-20C

Kit contents scale proportionally with size, except sealing tape.

Example: The V1 kit contains 5X the listed quantities above, but will exclude the sealing tape.

The microwell plate is supplied as 12 8-well modules - Each module is designed to break apart for 8 tests.

### Description

The PathScan® Hydroxy-HIF-1α (Pro564) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of hydroxy-HIF-1α (Pro564) protein. A HIF-1α antibody has been coated onto the microwells. After incubation with cell lysates, HIF-1α protein is captured by the coated antibody. Following extensive washing, a Hydroxy-HIF-1α (Pro564) Detection Antibody is added to detect the captured hydroxy-HIF-1α (Pro564) protein. Anti-rabbit IgG, HRP-linked antibody is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of optical density for this developed color is proportional to the quantity of hydroxy-HIF-1α (Pro564) protein. Antibodies in kit are custom formulations specific to kit.

### Specificity/Sensitivity

PathScan® Hydroxy-HIF-1α (Pro564) Sandwich ELISA Kit recognizes endogenous levels of hydroxy-HIF-1α (Pro564) protein, as shown in Figure 1. The kit sensitivity is shown in Figure 2. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

### Background

Hypoxia-inducible factor 1 (HIF1) is a heterodimeric transcription factor that plays a critical role in the cellular response to hypoxia (1). The HIF1 complex consists of two subunits, HIF-1α and HIF-1β, which are basic helix-loop-helix proteins of the PAS (Per, ARNT, Sim) family (2). HIF1 regulates the transcription of a broad range of genes that facilitate responses to the hypoxic environment, including genes regulating angiogenesis, erythropoiesis, cell cycle, metabolism, and apoptosis. The widely expressed HIF-1α is typically degraded rapidly in normoxic cells by the ubiquitin/proteasomal pathway. Under normoxic conditions, HIF-1α is proline hydroxylated leading to a conformational change that promotes binding to the von Hippel-Lindau protein (VHL) E3 ligase complex; ubiquitination and proteasomal degradation follows (3,4). Both hypoxic conditions and chemical hydroxylase inhibitors (such as desferrioxamine and cobalt) inhibit HIF-1α degradation and lead to its stabilization. In addition, HIF-1α can be induced in an oxygen-independent manner by various cytokines through the PI3K-AKT-mTOR pathway (5-7).

HIF-1β is also known as AhR nuclear translocator (ARNT) due to its ability to partner with the aryl hydrocarbon receptor (AhR) to form a heterodimeric transcription factor complex (8). Together with AhR, HIF-1β plays an important role in xenobiotics metabolism (8). In addition, a chromosomal translocation leading to a TEL-ARNT fusion protein is associated with acute myeloblastic leukemia (9).

Studies also found that ARNT/HIF-1 $\beta$  expression levels decrease significantly in pancreatic islets from patients with type 2 diabetes, suggesting that HIF-1 $\beta$  plays an important role in pancreatic  $\beta$ -cell function (10).

## Background References

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# #13201

## PathScan® Hydroxy-HIF-1α (Pro564) Sandwich ELISA Kit

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### ELISA Colorimetric

**NOTE:** Refer to product-specific datasheets or product webpage for assay incubation temperature.

### A. Solutions and Reagents

**NOTE:** Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L PBS: add 50 ml 10X PBS to 950 ml dH<sub>2</sub>O, mix.
2. Bring all microwell strips to room temperature before use.
3. Prepare 1X Wash Buffer by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in dH<sub>2</sub>O.
4. **1X Cell Lysis Buffer:** 10X Cell Lysis Buffer (#9803): To prepare 10 ml of 1X Cell Lysis Buffer, add 1 ml of 10X Cell Lysis Buffer to 9 ml of dH<sub>2</sub>O, mix. Buffer can be stored at 4°C for short-term use (1-2 weeks).

**Recommended:** Add 1 mM phenylmethylsulfonyl fluoride (PMSF) (#8553) immediately before use.

**NOTE:** Refer to product-specific datasheet or webpage for lysis buffer recommendation.

5. **TMB Substrate:** (#7004).
6. **STOP Solution:** (#7002).

### B. Preparing Cell Lysates

#### For adherent cells

1. Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
2. Remove media and rinse cells once with ice-cold 1X PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X cell lysis buffer plus 1 mM PMSF to each plate (10 cm diameter) and incubate the plate on ice for 5 min.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 min (x14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at -80°C in single-use aliquots.

#### For suspension cells

1. Remove media by low speed centrifugation (~1,200 rpm) when the culture reaches 0.5–1.0 x 10<sup>6</sup> viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1,200 rpm) and wash once with 5–10 ml ice-cold 1X PBS.
3. Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X cell lysis buffer plus 1 mM PMSF.
4. Sonicate lysates on ice.
5. Microcentrifuge for 10 min (x14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at -80°C in single-use aliquots.

### C. Test Procedure

1. After the microwell strips have reached room temperature, break off the required number of microwells. Place the microwells in the strip holder. Unused microwells must be resealed in the storage bag and stored at 4°C immediately.
2. Cell lysates can be undiluted or diluted with sample diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color). Individual datasheets or product webpage for each kit provide information regarding an appropriate dilution factor for lysates and kit assay results.
3. Add 100 µl of each undiluted or diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hr at 37°C. Alternatively, the plate can be incubated overnight at 4°C.
4. **Gently remove the tape and wash wells:**
  1. Discard plate contents into a receptacle.
  2. Wash 4 times with 1X wash buffer, 200 µl each time per well.
  3. For each wash, strike plates on fresh paper towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
  4. Clean the underside of all wells with a lint-free tissue.
5. Add 100 µl of detection antibody (green color) to each well. Seal with tape and incubate the plate at 37°C for 1 hr.

6. Repeat wash procedure (Section C, Step 4).
7. Add 100  $\mu$ l of HRP-linked secondary antibody (red color) to each well. Seal with tape and incubate the plate for 30 min at 37°C.
8. Repeat wash procedure (Section C, Step 4).
9. Add 100  $\mu$ l of TMB substrate to each well. Seal with tape and incubate the plate for 10 min at 37°C or 30 min at 25°C.
10. Add 100  $\mu$ l of STOP solution to each well. Shake gently for a few seconds.

**NOTE:** Initial color of positive reaction is blue, which changes to yellow upon addition of STOP solution.

**11. Read results**

1. **Visual Determination:** Read within 30 min after adding STOP solution.
2. **Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP solution.

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