

# HIF2- $\alpha$ Recombinant Monoclonal Antibody [BL-95-1A2]

Rabbit Recombinant Monoclonal

Purified Protein ID NP\_001421.2  
Catalog No. A700-003 GeneID 2034  
Lot No. A700-003-3



**APPLICATIONS** WB, IP, IHC, ICC, ICC-IF, ChIP-Seq  
**SPECIES REACTIVITY** Human  
**AMOUNT** 100  $\mu$ l (10 blots)  
**CONCENTRATION** 400  $\mu$ g/ml  
**STORAGE/SHELF LIFE** 2 - 8 $^{\circ}$  C / 1 year from date of receipt  
**PHYSICAL STATE** Liquid  
**BUFFER** Borate Buffered Saline (BBS) pH 8.2 with 0.1% BSA and 0.09% Sodium Azide  
**ISOTYPE** IgG  
**CLONE #** BL-95-1A2  
**ORIGIN** USA

**PRODUCTION PROCEDURES** Recombinant antibody was purified from cell culture supernatant.

The epitope recognized by A700-003 maps to a region between residues 400 and 450 of human endothelial PAS domain protein 1 using the numbering given in entry NP\_001421.2 (GeneID 2034).

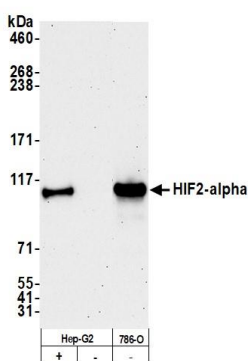
**APPLICATIONS** Centrifuge tube to remove product from lid. Optimal working dilutions should be determined experimentally by the investigator. Prepare working dilution immediately before use.

Western Blot 1:1000  
Immunoprecipitation 5 - 20  $\mu$ l/mg lysate  
Immunohistochemistry 1:200 - 1:1,000. Epitope retrieval with citrate buffer pH 6.0 for 20 minutes using a pressure cooker is recommended for FFPE tissue sections. Overnight incubations are suggested.  
Immunocytochemistry 1:200 - 1:1,000. Formaldehyde fixation is recommended. Permeabilization with Triton-X 100 is recommended for formaldehyde-fixed cells.  
Immunofluorescence (ICC) 1:100 - 1:500. Formaldehyde fixation is recommended. Permeabilization with Triton-X 100 is recommended for formaldehyde-fixed cells.  
ChIP-Seq A previous lot has qualified for ChIP-Seq. 10 - 40  $\mu$ l per 30  $\mu$ g chromatin.

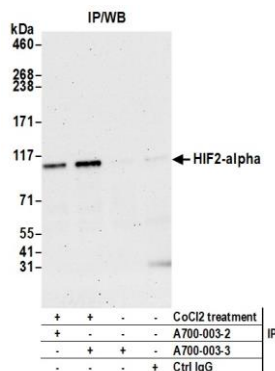
**IHC HUMAN CONTROLS** Renal Cell Carcinoma, 786-O Cells, Hep-G2 Cells

**ADDITIONAL INFO** <https://www.bethyl.com/product/A700-003>  
Use the link above to view SDS, a current list of citations, and other product specific information.

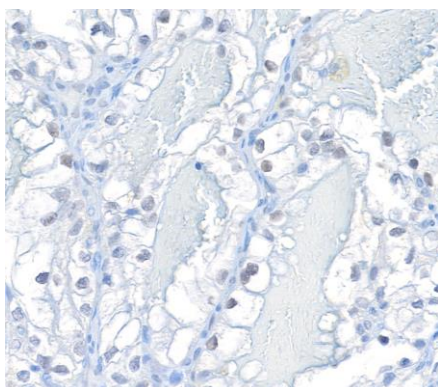
This document certifies that this product has met all of the quality control standards defined by Bethyl Laboratories, Inc.  
Brian McWilliams, PhD Date: June 3, 2021



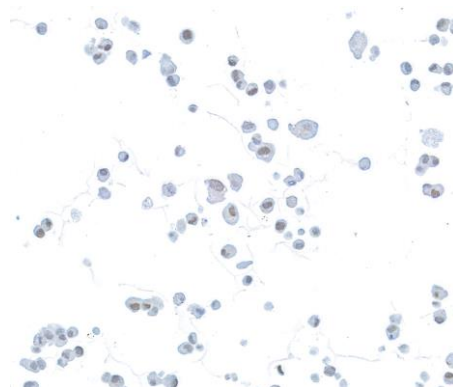
**Detection of human HIF2-alpha by western blot. Samples:** Whole cell lysate (50 µg) from HepG2 and 786-O cells treated with 200 µM CoCl<sub>2</sub> (+) or mock treated (-). **Antibody:** Rabbit anti-HIF2-alpha recombinant monoclonal antibody [BL-95-1A2] (A700-003 lot 3) used at 1:1000. **Secondary:** HRP-conjugated goat anti-rabbit IgG (A120-101P). Chemiluminescence with an exposure time of 75 seconds.



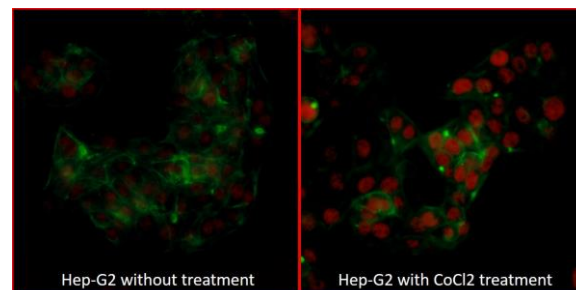
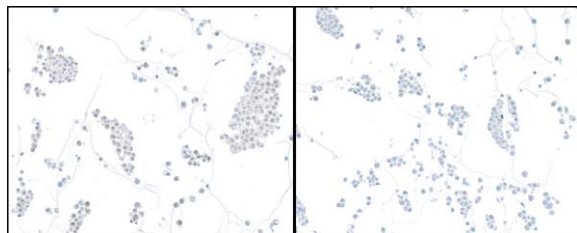
**Detection of human HIF2-alpha by western blot of immunoprecipitates. Samples:** Whole cell lysate (1.0 mg per IP reaction; 20% of IP loaded) from Hep-G2 cells with 200 µM CoCl<sub>2</sub> (+) or mock treated (-). **Antibodies:** Rabbit anti-HIF2-alpha recombinant monoclonal antibody [BL-95-1A2] (A700-003 lot 3) used for IP at 20 µl/mg lysate. HIF2-alpha was also immunoprecipitated by a previous lot of this antibody (A700-003 lot 2). For blotting immunoprecipitated HIF2-alpha, A700-003 was used at 1:1000. Chemiluminescence with an exposure time of 75 seconds.



**Detection of human HIF2-alpha in FFPE renal cell carcinoma by IHC. Antibody:** Rabbit anti-HIF2-alpha recombinant monoclonal antibody [BL95-1A2] (A700-003 lot 3). **Secondary:** HRP-conjugated goat anti-rabbit IgG (A120-501P). **Substrate:** DAB.



**Detection of human HIF2-alpha in FFPE 786-O cells by ICC. Antibody:** Rabbit anti-HIF2-alpha recombinant monoclonal antibody [BL95-1A2] (A700-003 lot 3). **Secondary:** HRP-conjugated goat anti-rabbit IgG (A120-501P). **Substrate:** DAB.

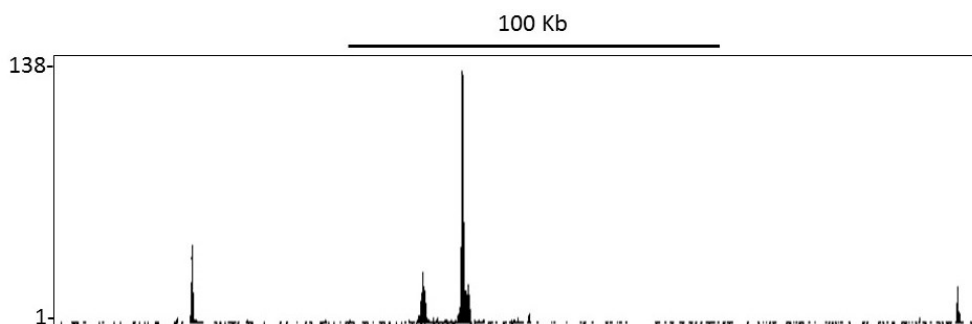


**Detection of human HIF2-alpha in FFPE Hep-G2 cells with (left) and without (right) cobalt treatment by ICC.**

*Antibody:* Rabbit anti-HIF2-alpha recombinant monoclonal antibody [BL95-1A2] (A700-003 lot 3). *Secondary:* HRP-conjugated goat anti-rabbit IgG (A120-501P). *Substrate:* DAB.

**Detection of human HIF2-alpha by immunocytochemistry.**

*Sample:* Formaldehyde-fixed Hep-G2 cells untreated (left) and treated with CoCl<sub>2</sub> (right). *Antibody:* Rabbit anti-HIF2-alpha recombinant monoclonal antibody [BL-95-1A2] (A700-003 lot 2) used at 1:100. *Secondary:* DyLight® 594-conjugated goat anti-rabbit IgG (A120-201D4) used at 1:100. *Counterstain:* Phalloidin Alexa Fluor® 488 conjugated (green).



**Localization of HIF2-alpha Binding Sites by ChIP-seq.** Chromatin from subcutaneous human tumor 786-O cells was immunoprecipitated with anti-HIF2-alpha antibody A700-003 and analyzed by DNA sequencing. The figure illustrates the peak distribution of HIF2-alpha binding within a 250 Kb region of chromosome 11 as detected using anti-HIF2-alpha antibody A700-003. ChIP-seq validation performed by Active Motif, Carlsbad, CA.