APA798Hu02 100μg
Active Hypoxia Inducible Factor 1 Alpha (HIF1a)
Organism Species: Homo sapiens (Human)
Instruction manual

FOR IN VITRO USE AND RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1th Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: Thr218~Thr506
Tags: N-terminal His-tag
Purity: >95%
Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 5% trehalose.
Applications: Cell culture; Activity Assays; In vivo assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 5.0
Predicted Molecular Mass: 35.9kDa
Accurate Molecular Mass: 40kDa as determined by SDS-PAGE reducing conditions.
Phenomenon explanation:
The possible reasons that the actual band size differs from the predicted are as follows:

1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
2. Relative charge: The composition of amino acids may affects the charge of the protein.
3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
5. Polymerization of the target protein: Dimerization, multimerization etc.

[USAGE]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

TCL VLICEPIPHP SNIEIPLDSK TFLSRHSLDM
KFSYCDERIT ELMGYEPEEL LGRSIYEYYH ALDSDHLTKT HHDMFTKGQV
TTGQYRMLAK RGGYVWVETQ ATVIYNTKNS QPQCIVCVNY VVSGIIQHDL
IFSLQQTECV LKPVESSDMK MTQLFTKVES EDTSSLFDKL KKEPDALTLL
APAAGDTIIS LDFGSNDTET DDQQLEEVPL YNDVMLPSPN EKLQNINLAM
SPLPTAETPK PLRSSADPAL NQEVALKLEP NPESLELSFT MPQIQDQTPS
PSDGST

[ACTIVITY]

HIF1a (Hypoxia-inducible factor 1-alpha) is a subunit of a transcription factor, which functions as a master transcriptional regulator of the adaptive response to hypoxia. The protein chaperone heat shock protein 90 (Hsp90) is a major regulator of different transcription factors, including HIF1a, thus a binding ELISA assay was conducted to detect the interaction of HIF1a and HSP90. Briefly, recombinant human HIF1a were diluted serially in PBS, with 0.01%BSA (pH 7.4). Duplicate samples of 100uL were then transferred to HSP90-coated microtiter wells and

incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-HIF1a pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37°C. Finally, add 50μL stop solution to the wells and read at 450nm immediately. The binding activity of of HIF1a and HSP90 was shown in Figure 1, and this effect was in a dose dependent manner.

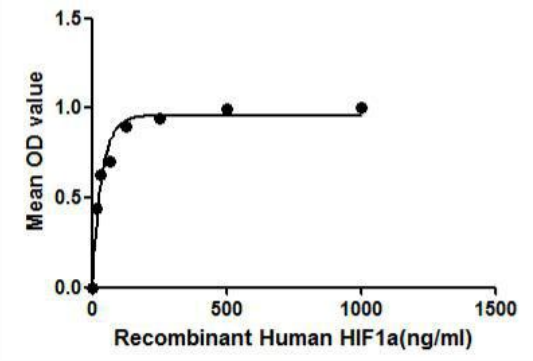

Figure 1. The binding activity of HIF1a with HSP90.

[IDENTIFICATION]

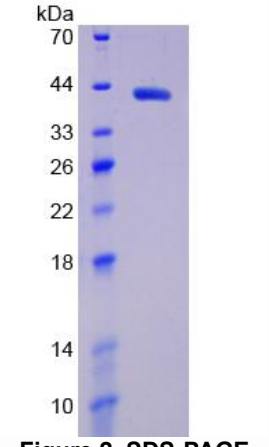

Figure 2. SDS-PAGE

Sample: Active recombinant HIF1a, Human

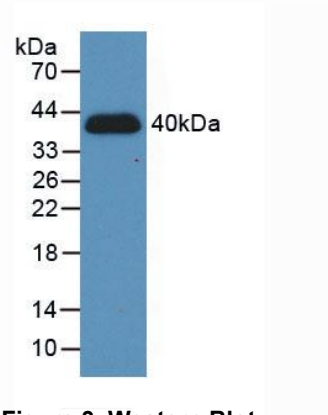

Figure 3. Western Blot

Sample: Recombinant HIF1a, Human;

Antibody: Rabbit Anti-Human HIF1a Ab (PAA798Hu02)