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APP948Hu01 100µg Active Homeobox Protein D13 (HOXD13) Organism Species: *Homo sapiens* (Human) *Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression. Host: E. coli Residues: Met1~Ser343 Tags: N-terminal His-tag **Purity:** >80% **Endotoxin Level:** <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: PBS, pH7.4, containing 0.01% Sarcosyl, 5% Trehalose. Original Concentration: 200µg/mL Applications: Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 9.8 Predicted Molecular Mass: 39.8kDa Accurate Molecular Mass: 44kDa 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.

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[<u>USAGE</u>]

Reconstitute in 10mM PBS (pH7.4) 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.

[<u>SEQUENCE</u>]

MSRAGSWDMDGLRADGGGAGGAPASSSSSVAAAAASGQCRGFLSAPVFA GTHSGRAAAAAAAAAAAAAAASGFAYPGTSERTGSSSSSSSAVVAARPEAPP AKECPAPTPAAAAAAPPSAPALGYGYHFGNGYYSCRMSHGVGLQQNALKSSP HASLGGFPVEKYMDVSGLASSSVPANEVPARAKEVSFYQGYTSPYQHVPGYID MVSTFGSGEPRHEAYISMEGYQSWTLANGWNSQVYCTKDQPQGSHFWKSS FPGDVALNQPDMCVYRRGRKKRVPYTKLQLKELENEYAINKFINKDKRRRISAA TNLSERQVTIWFQNRRVKDKKIVSKLKDTVS

[ACTIVITY]

Homeobox protein Hox - D13 (HOXD13) is a key transcription factor in the homeobox family.HOXD13 is crucial for tissue and organ patterning and differentiation, especially in axial skeleton and limb development. Abnormalities in HOXD13, like mutations or abnormal expressions, can cause developmental disorders such as synpolydactyly, highlighting its importance in normal morphological development.

Besides, Hoxd-13 can bind to and transactivate the Shh promoter in vitro, thus a

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functional binding ELISA assay was conducted to detect the interaction of recombinant human HOXD13 and recombinant human SHH .Briefly, biotin-linked HOXD13 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100 μ I were then transferred to SHH-coated microtiter wells and incubated for 1h at 37 °C. Wells were washed with PBST 3 times and incubation with Streptavidin-HRP for 30min, then wells were aspirated and washed 5 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 HOXD13 and SHH was shown in Figure 1, the EC50 for this effect is 0.019ug/mL.

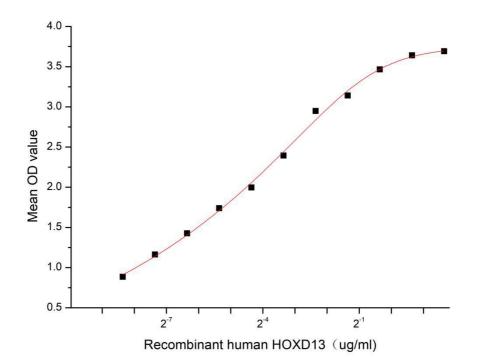


Figure 1. The binding activity of recombinant human HOXD13 and recombinant human

SHH

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[IDENTIFICATION]

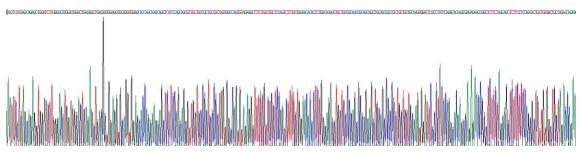


Figure 2. Gene Sequencing (extract)

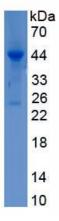


Figure 3. SDS-PAGE

Sample: Active recombinant HOXD13, Human

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.