SANTA CRUZ BIOTECHNOLOGY, INC.

His-probe (G-18): sc-804

BACKGROUND

Plasmid vectors for the expression of coding regions of eukaryotic genes in bacterial, insect and mammalian hosts are in common usage; such expression vectors are frequently used to encode hybrid fusion proteins consisting of a eukaryotic target protein and a specialized region designed to aid in the purification and visualization of the target protein. A system that has proven to be very successful relies on the insertion of a six histidine (His6) sequence in the N-terminus of the encoded protein, allowing for efficient coupling to Ni^{2+}-chelating resins and purification by single step affinity chromatography. This polyhistidine sequence can then be removed by specific cleavage at sites recognized by enzymes such as thrombin or enterokinase, permitting the separation of the target protein from the polyhistidine tag. Visualization of such fusion proteins can be achieved by utilizing antibodies generated against specific peptide sequences downstream from the multiple cloning site.

REFERENCES

1. Maniattis, T., et al. 1982. Molecular Cloning. Cold Spring Harbor, New York: Cold Spring Laboratory.
2. Smith, D.B., et al. 1988. Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. Gene 67: 31-40.
3. Hochuli, E. 1988. Large-scale chromatography of recombinant proteins. J. Chromatogr. 444: 293-302.

SOURCE

His-probe (G-18) is available as either rabbit (sc-804) or goat (sc-804-G) polyclonal affinity purified antibody raised against a peptide mapping of His-probe.

PRODUCT

Each vial contains 100 µg IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Blocking peptide available for competition studies, sc-804 P, (100 µg peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% BSA).

APPLICATIONS

His-probe (G-18) is recommended for detection of fusion proteins encoded by pET expression vectors with polyhistidine sequence domains origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) and immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)].

STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

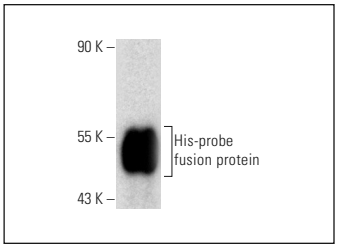
PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.

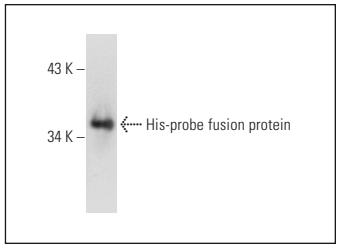
RESEARCH USE

For research use only, not for use in diagnostic procedures.

DATA



His-probe (G-18): sc-804. Western blot analysis of mouse recombinant RIP140 fusion protein.



His-probe (G-18)-G: sc-804-G. Western blot analysis of mouse recombinant His-probe tagged GRIP1 fusion protein.

SELECT PRODUCT CITATIONS

1. Levchenko, I., et al. 1997. PDZ-like domains mediate binding specificity in the Clp/Hsp100 family of chaperones and protease regulatory subunits. Cell 91: 939-947.
2. Sung, M., et al. 2010. Biological characterization and structure based prediction of Insulin-like growth factor binding protein-5. Biochem. Biophys. Res. Commun. 403: 230-236.
3. Ishiyama, S. and Ikeda, M. 2010. High-level expression and improved folding of proteins by using the vp39 late promoter enhanced with homologous DNA regions. Biotechnol. Lett. 32: 1637-1647.
4. Hui, S., et al. 2011. Peptide-mediated disruption of calmodulin-cyclin E interactions inhibits proliferation of vascular smooth muscle cells and neointima formation. Circ. Res. 108: 1053-1062.
5. Girard, G. and Rigali, S. 2011. Role of the phenazine-inducing protein Pip in stress resistance of *Pseudomonas chlororaphis.* Microbiology 157: 398-407.
6. Wang, W.M., et al. 2011. Binding site specificity and factor redundancy in activator protein-1-driven human papillomavirus chromatin-dependent transcription. J. Biol. Chem. 286: 40974-40986.
7. Motohashi, H., et al. 2011. Molecular determinants for small Maf protein control of platelet production. Mol. Cell. Biol. 31: 151-162.
8. Lee, J.Y., et al. 2011. Human HOXA5 homeodomain enhances protein transduction and its application to vascular inflammation. Biochem. Biophys. Res. Commun. 410: 312-316.