



His-probe (16-002): sc-65903

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 an 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 Ni²⁺-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

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3. Hochuli, E. 1988. Large-scale chromatography of recombinant proteins. *J. Chromatog.* 444: 293-302.
4. Thanos, D. and Maniatis, T. 1992. The high mobility group protein HMG I(Y) is required for NFκB-dependent virus induction of the human IFN-β gene. *Cell* 71: 777-789.
5. Kroll, D.J., Abdel-Hafiz, H., Marcell, T., Simpson, S., Chen, C.Y., Gutierrez-Hartmann, A., Lustbader, J.W. and Hoeffler, J.P. 1993. A multifunctional prokaryotic protein expression system: overproduction, affinity purification, and selective detection. *DNA Cell Biol.* 12: 441-453.
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SOURCE

His-probe (16-002) is a mouse monoclonal antibody raised against a 6x His-tagged homooligopeptide.

PRODUCT

Each vial contains 100 µg IgG₁ in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

STORAGE

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

RESEARCH USE

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

APPLICATIONS

His-probe (16-002) is recommended for detection of linear HHH HHH origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) and immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

PROTOCOLS

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