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Detection of histidine-tagged fusion proteins by using a high-specific mouse monoclonal anti-histidine tag antibody

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Recombinant proteins are utilized for various purposes in molecular biology such as the production of antibodies or investigation of the mechanism of protein–nucleic acid or protein–protein interactions. Many prokaryotic expression vectors have been established enabling synthesis of the protein of interest as a fusion with a peptide, thus facilitating purification. Expression of recombinant proteins in *Escherichia coli* as a fusion protein with neighbouring histidine residues is one of the most popular methods (1; Diagen), because these proteins have useful attributes. The affinity of the histidine tag motif to Ni^{2+} ions by chelation is strong and selective enough to enable purification of the protein to homogeneity by affinity chromatography on a Ni^{2+}-NTA adsorbant (1). Additionally, an enterokinase cleavage site engineered between the N- or C-terminus of the protein of interest and the polyhistidine fusion partner facilitates the removal of the tag. A drawback of the polyhistidine fusion system so far may be the lack of sera specific for the affinity tag. To circumvent this limitation we raised mouse monoclonal anti-histidine tag antibodies by immunizing mice with mixtures of histidine-tagged proteins, engineered in our laboratory using standard immunizing protocols (2). Such an antibody allows the detection of proteins with a histidine tag without the need to raise specific sera against the protein of interest. Here we present data obtained using the anti-histidine tag antibody mAb 13/45/31 (subclass IgG2a).

First we tested mAb 13/45/31 in immunofluorescence analysis. For this we transfected p53-negative SAOS-2 cells with the expression plasmid pUHD-His-p53mt267. This plasmid is a derivative of plasmid pUHD 10-1 (3) with an additional six histidine residues and an enterokinase cleavage site at the N-terminus of p53 coding region. Staining with DAPI (4′,6-diamidine-2′-phenylindole dihydrochloride; Boehringer) allowed detection of cell nuclei (Fig. 1a). Most of the cells expressed the p53 histidine fusion protein clearly demonstrated by immunofluorescence with mAb 13/45/31 (Fig. 1b) or rabbit anti-p53-serum (Fig. 1c). With both antibodies the identical staining pattern of cell nuclei was observed. In another set of experiments we performed immunoblot analyses using mAb 13/45/31. Lysates of bacteria transformed with pQE/HBx comprising the X reading frame of the hepatitis B virus and an enterokinase cleavage site cloned into pQE-8 were analyzed before and after induction with IPTG (isopropyl-β-D-thiogalactopyranoside; Serva) by polyacrylamide gel electrophoresis (PAGE) and Coomassie-blue staining (Fig. 2, lanes 1 and

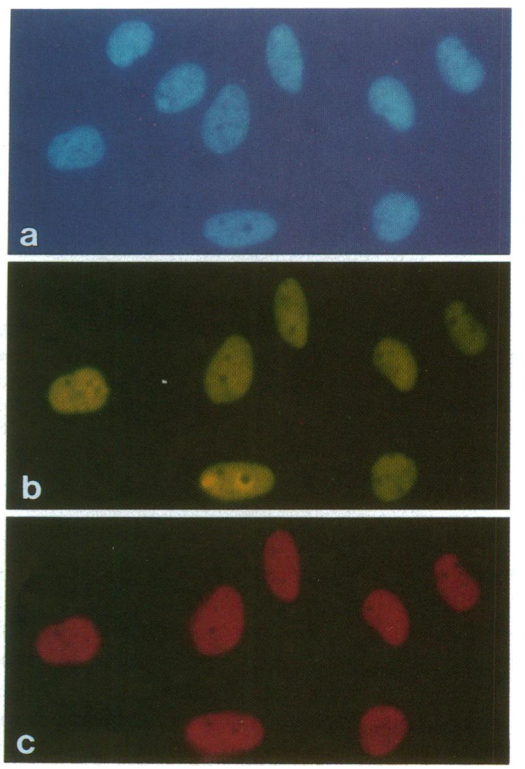

Figure 1. Immunofluorescence of SAOS-2 cells transfected with the expression plasmid pUHD-His-p53mt267. Cells were stained simultaneously with (**a**) DAPI, (**b**) mAb 13/45/31 as first and a FITC coupled goat–anti-mouse antibody as secondary antibody and (**c**) a polyclonal rabbit anti-p53 serum and, subsequently, a Cy3 coupled goat–anti-rabbit antibody.

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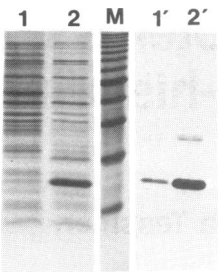

Figure 2. Detection of HBx protein with N-terminal histidine-tag and enterokinase cleavage site in bacterial lysates after PAGE and Coomassie-blue staining (lanes 1 and 2) and immunoblotting of a gel run in parallel with the mAb 13/45/31 (lanes 1′ and 2′). Samples were analyzed before (lanes 1 and 1′) and after IPTG induction of the bacteria (lanes 2 and 2′). Marker, M; 10 kDa ladder (Gibco-BRL).

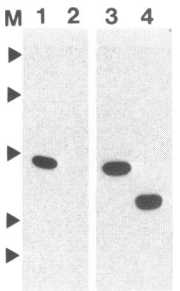

Figure 3. Detection of purified recombinant Cofilin expressed as fusion protein tagged at the N-terminus with an enterokinase site and six histidine residues. Immunoblotting was performed before (lanes 1 and 3) and after cleavage with enterokinase (lanes 2 and 4) with mAb 13/45/31 (lanes 1 and 2) and a polyclonal rabbit anti-Cofilin antibody (lanes 3 and 4). Marker positions are 15, 18, 30, 45 and 67 kDa.

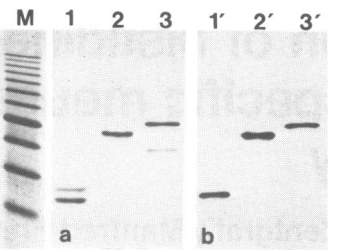

Figure 4. Detection of purified recombinant fusion proteins tagged at the C-terminus with six histidine residues by PAGE and Coomassie-blue staining (**a**) and immunoblotting (**b**) with mAb 13/45/31. Proteins were C22 (lanes 1 and 1′), C33 (lanes 2 and 2′) and Ba (lanes 3 and 3′). Marker, M; 10 kDa ladder with lowest band 20 kDa.

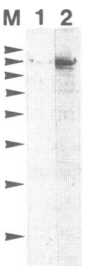

Figure 5. Immunoprecipitation of recombinant histidine and enterokinase site tagged fusion protein hsp70 from bacterial extracts with mAb 13/45/31. Precipitation was performed with bacterial lysate before (lane 1) and after (lane 2) induction and analyzed following PAGE and immunoblotting with a polyclonal rabbit anti-hsp70 serum. Marker, M; 10 kDa ladder.

2). The ~18 kDa fusion protein was clearly detectable after IPTG induction (Fig. 2, lane 2). The corresponding immunoblot is shown in Figure 2 (lanes 1′ and 2′) and documents the high specificity of mAb 13/45/31. Even low amounts of protein not attributable to the recombinant protein after Coomassie-blue staining were detectable after immunoblotting with the mAb 13/45/31 (Fig. 2, lanes 1 and 1′). Cleavage of the N-terminal-tagged histidine–enterokinase–fusion protein with enterokinase (Boehringer) prior to immunoblotting resulted in the complete loss of the specific signal of mAb 13/45/31 as shown for the Cofilin fusion protein (Fig. 3, compare lanes 1 and 2). A blot prepared in parallel using a polyclonal rabbit anti-Cofilin serum served as a control (Fig. 3, lanes 3 and 4). In a further assay we performed PAGE followed by immunoblotting with purified fusion proteins tagged solely with six histidine residues at the C-terminus. After Coomassie-blue staining (Fig. 4a) the pattern of the proteins C22 (lanes 1 and 1′), C33 (lanes 2 and 2′), and Ba (lanes 3 and 3′) was identical to that observed after immunoblotting using mAb 13/45/31 (Fig. 4b) thus demonstrating the highly specific affinity of mAb 13/45/31 to the epitope formed by the six histidine residues. To prove further applicabilities of mAb 13/45/31 we performed immunoprecipitation studies. One example is given in Figure 5. Bacteria were transformed with a histidine–enterokinase–heat shock protein (hsp70) construct and expression was induced with IPTG. Using the mAb 13/45/31 we performed immunoprecipitation with bacterial lysates before and after induction. After PAGE of the precipitate and immunoblotting with a polyclonal rabbit anti-hsp70 serum we detected the precipitated hsp70 fusion protein at the expected mol. wt of ~70 kDa. Other applications we have successfully examined are the use of mAb 13/45/31 in ELISA and microinjection studies (data not shown). From the data presented here, using mAb 13/45/31 in immunofluorescence, immunoblotting and immunoprecipitation studies and, in addition, in analysis using ELISA and microinjection techniques, we believe mouse monoclonal anti-histidine tag antibody 13/45/31 is a powerful tool in molecular and cellular biology.

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