

Specific applications

Direct immunofluorescent detection of an HA-tagged protein in yeast

Dr. Susan Michaelis of The Johns Hopkins University School of Medicine has used **Anti-HA-rhodamine** to directly stain HA-tagged STE6 gene product, a membrane protein required for the transport of the **a-factor** mating pheromone in *S. cerevisiae*. Details of the construction of the amino- or carboxy-terminally tagged STE6 proteins have been published (Berkower, Loayza, and Michaelis, 1994).

Briefly, three copies of the HA-epitope were added by recombinant methods to the amino-terminal or to the carboxy-terminal region of the STE6 gene on a yeast shuttle vector. Each vector containing HA-tagged STE6 was introduced into a yeast strain by lithium acetate transformation. Cells were prepared and directly stained with Anti-HA-fluorescein as described in Procedure II above. Figures 3B.2 and 3B.3 show typical results.

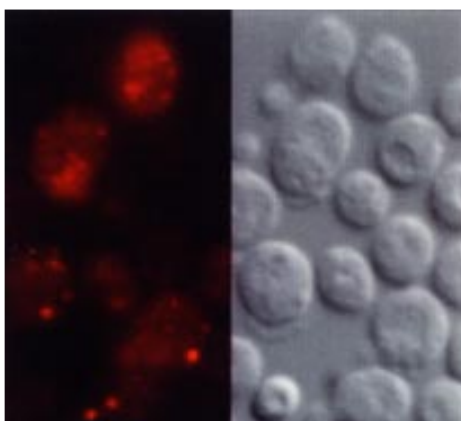


Figure 3B.2: Direct immunofluorescent detection of HA-tagged STE6 in yeast cells. Cells were prepared, fixed, and stained with Anti-HA-rhodamine. The right panel is a phase-contrast photomicrograph of all cells in the field. The left panel shows the immunofluorescently stained cells with the tagged wild type STE6 gene product (red dots) localized in the Golgi. (Photo from G. Nijbroek and S. Michaelis; reprinted by permission).

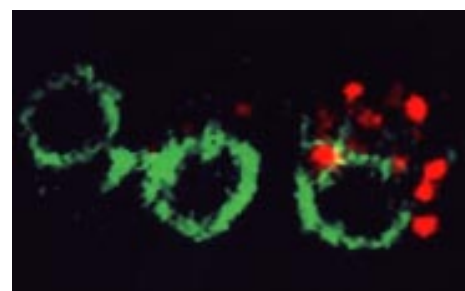


Figure 3B.3: Confocal image of yeast cells containing HA-tagged STE6. Cells were prepared, fixed, stained, and analyzed by confocal fluorescence microscopy. The deconvoluted image shows HA-tagged STE6 (red, stained with Anti-HA-rhodamine) and Kar2, an endoplasmic reticulum marker (green, stained with a fluorescein-conjugated antibody). (Photo from G. Nijbroek and S. Michaelis; reprinted by permission).

Indirect immunofluorescent detection of an HA-tagged protein in a transiently transfected human cell line

Dr. Jürgen Wess of the National Institutes of Health has used **Anti-HA** monoclonal antibody to analyze a set of HA-tagged domains from the rat m3 muscarinic acetylcholine receptor as they were transiently expressed in COS-7 cells. Details of the procedure have been published (Schöneberg, Liu and Wess, 1995).

Briefly, for each tagged domain, the HA epitope and a stop codon were added to the mammalian expression vector Rm3pcD (which contains the entire coding sequence of the rat m3 muscarinic receptor) by PCR. Each resulting construct, encoding either HA-tagged amino-terminal or carboxy-terminal receptor domain, was transfected into growing COS-7 cells. One day after the transfection, cells were transferred to a coverslip in one well of a six-well plate, fixed with 4% formaldehyde, permeabilized with 0.5% Triton X-100 (optional), and stained with **Anti-HA** monoclonal antibody (10 $\mu\text{g}/\text{ml}$, 2 h, 37°C). The antigen-antibody complexes were stained with a 100- or 200-fold dilution of goat Anti-mouse IgG-fluorescein (1 h, 37°C). Figure 4B.4 shows a typical result.

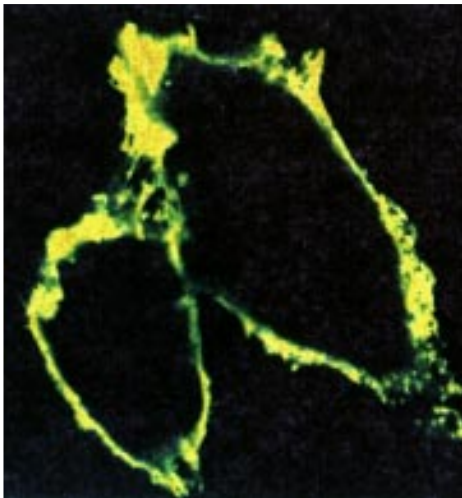


Figure 3B.4: Indirect immunofluorescent detection of a HA-tagged domain from the m3 muscarinic receptor expressed in COS-7 cells. The domain was tagged at the amino-terminus. The cells in this image were not permeabilized with Triton X-100 prior to staining. The image was obtained with a confocal laser-scanning microscope. [From Schöneberg, Liu, and Wess (1995); reprinted by permission of the Journal of Biological Chemistry].

References

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