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Product specifications

ACIDIC GRANULE KIT

Product # D 09 Typical Lot

DESCRIPTION OF KIT CONTENTS:

DAMP: [3-(2,4-dinitroanilino)-3'-amino-N-methyldipropylamine][1]

Quantity: 1 mg of the hydrobromide salt in 1 mL of 80% ethanol.

Purity: Greater than 98%

Stability: >Two years at –80°,
NOTE: Protect from light.

Monoclonal Anti-DNP [clone HDP1][2]

Quantity: Approximately 0.8 mg of protein A purified IgG in 1mL phosphate buffered saline containing 0.1% sodium azide.

Stability**:** > One year below 10°C.

BACKGROUND:

The DAMP method for cytochemical visualization of acidic compartments offers advantages over the use of vital pH indicators in visualizing acidic compartments in that it allows finer resolution as it can be used in conjunction with light or electron microscopy. DAMP, when incubated with viable cell, accumulates in acidic compartments. The location of the acidic compartments may then be visualized by immunofluorescence after fixation by treatment with monoclonal anti-DNP, followed by a fluorescent labeled secondary antibody. Alternatively, one may visualize the acidic

compartments by light or immunoelectron microscopy using a peroxidase labeled secondary antibody. For immunoelectron microscopy, protein A-immunogold labeling is recommended. These methods have already been successfully used to study low pH compartments in a number of cell types.

EXPERIMENTAL PROCEDURES:

Although some variations in technique may be required for different cell types, the following methods should generally yield acceptable results.

A. Incorporation of DAMP into cells:

Incubate the cells with 30 µM DAMP for at least 30 minutes. A 1:100 final dilution of solution A is therefore required. For example, add 20 µL of solution A to the cells in 2 mL of culture medium and incubate at 37° for 30 minutes.

B. Indirect Immunofluorescence:

1. FIXATION: Fix cells at room temperature for 15 minutes with 3% (wt/vol) paraformaldehyde in buffer C (10 mM sodium phosphate, 150 mM sodium chloride, 2 mM magnesium chloride, pH 7.4). Then wash them once with 50 mM ammonium chloride and twice with buffer C. The cells must them be permeabilized by incubation with 0.1% Triton X-100 in buffer C for 5 minutes at –10°C.
2. INCUBATION WITH ANTI-DNP: Incubate the fixed cells in a minimal volume with a 1:10 final dilution of kit component B (monoclonal anti-DNP) for 60 minutes at 37°C. This corresponds to a final concentration of the antibody of approximately 80 µg/mL. The fixed cells are subsequently washed four times with buffer C to remove unbound IgG.
3. FLUORESCENT TAGGING: The cells are then incubated for 60 minutes at 37°C. with at least 60 µg/mL of antimouse IgG conjugated to an appropriate fluorescent label (eg. FITC or rhodamine) – NOT SUPPLIED WITH THIS KIT – and viewed under a fluorescent microscope.

C. Immunoelectron Microscopy:

1. FIXATION: Fix cells in 2.0% paraforamldehyde in buffer D (10 mM sodium periodate, 0.75 M lysine, 37.5 mM sodium phosphate, pH 6.2)[1]. Alternately, cells can be fixed with 1% glutaraldehyde in calcium and magnesium free Dulbecco's phosphate-buffered saline (PBS), pH 7.2 for 1 hour at 25°C. The cells are then washed once in the fixative and incubated in 0.5 M ammonium chloride in PBS for 30 minutes at 25°C.

2. INDIRECT IMMUNOPEROXIDASE LABELING: Process the fixed cells for indirect immunoperoxidase staining of intracellular sites by the method of Louvard *et al* [6]. This is done in a minimal volume with a 1:10 final dilution of kit component B (monoclonal anti-DNP), which corresponds to a final concentration of the antibody of approximately 80 µg/mL. Horseradish peroxidase-conjugated antimouse-IgG is used at a final concentration of 0.5 mg/mL. After washing to remove unbound HRP-antimouse-IgG, the HRP is visualized by incubation of the cells at room temperature for 10 minutes with 0.2% (w/v) diaminobenzidine in 0.01% H_2O_2. The cells are subsequently fixed with 2% osmium tetroxide and 1% potassium ferrocyanide in 0.1 M cacodyate (pH 7.3), dehydrated and embedded for sectioning.

3. IMMUNOGOLD STAINING: For quantification of acidic granules, protein A-gold or IgG gold procedures are recommended. For details of these procedures see Anderson and Pathak[3].

REFERENCES:

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