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Anti-Bovine Respiratory Syncytial Virus Monoclonal Antibody labelled with Fluorescein Isothiocyanate

BIO 032

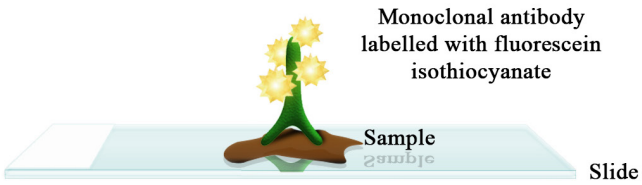
Reagent for direct immunofluorescence

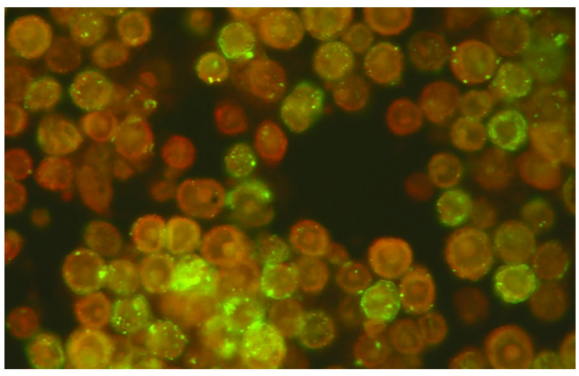
REAGENT FOR DETECTION OF BRSV ON TISSUE SECTION OR CELL CULTURE

INTRODUCTION

In Europe, BRSV is the most important aetiological agent responsible for respiratory affections in young cattle. In cattle as in children, respiratory syncytial viruses can cause a very deep attack of the respiratory tree. The infections often cause very severe injuries, which are responsible for high economic losses. As a matter of fact, in Europe, 7 million calves contract infectious diseases yearly. Sixty percent of these cases involve respiratory pathogens, and 1 million calves actually die of respiratory diseases each year in the European Community. The cost of these diseases, including medical treatments, growth delays and mortality, is about 450 millions EURO per year for calves under one year of age. For dairy cattle, the cost of BRSV has been evaluated at around 25 EURO per animal. BRSV principally affects young cattle. Beef cattle are especially vulnerable because of the animals' very high muscle mass compared with the pulmonary volume. The clinical manifestations of the infections can be dramatic. Signs of severe pneumonia such as polypnoea, abdominal bleeding, and hyperthermia are often present. Reinfections can be observed but usually remain subclinical. Clinical diagnosis is very difficult and laboratory assistance is required for a precise diagnosis. The virus can be detected in lung tissue by fluorescein-labelled specific antibodies. The direct immunofluorescence assay enables one to detect the presence of BRSV in frozen tissue sections made from lung tissue fragments (preferable from the cranioventral lobes, at the boundary between the diseased and apparently health tissue). The virus rarely occurs in the posterior lobes, in which emphysematous lesions are more frequently found. The reagent can be used to identify BRSV inside cells obtained by swabbing the pituitary mucosa (nasal swab). It can also be used to identify the virus's presence on an infected cell culture.

EXAMPLE OF RESULTS





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Fix the cell preparation (cell cultures or tissue sections) for 15 minutes at room temperature with one of the following fixators :

- Paraformaldehyde 2 % in PBS
- Acetone solution (9 volumes of acetone and 1 volume of water).
- Isopropanol
- Ethanol

Rince with PBS.

Dilute the conjugate twentyfold with a PBS-Evans blue solution made up according to the following formula:

PBS - Blue Evans

NaCl:	8 gr
KH2PO4:	0.2 gr
KCl:	0.2 gr
Na2HPO4 . 2H2O:	1.15 gr
Blue Evans:	0.01 gr
NaN3:	0.1 gr
H20	1 L

Incubate the sample with the fluorescein-labelled conjugate for 1 hour at room temperature. At the end of this incubation period rinse the cell preparation with a PBS solution. Dry the cell preparation, then add the mounting medium prepared as follows:

Mounting medium

Glycerol	9 volumes
PBS	1 volume

Examine the cell preparation under a microscope equipped for detecting fluorescence.

COMPOSITION: One vial of 500 µl

STORING THE CONJUGATE: The conjugate must be stored at 4°C. It must never be frozen.

STABILITY: One year at 4°C

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