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Anti-*Lawsonia intracellularis* Monoclonal antibody

BIO 323

Reagent for indirect immunofluorescence or immunoperoxidase

REAGENT FOR DETECTION OF LAWSONIA INTRACELLULARIS ON TISSUE

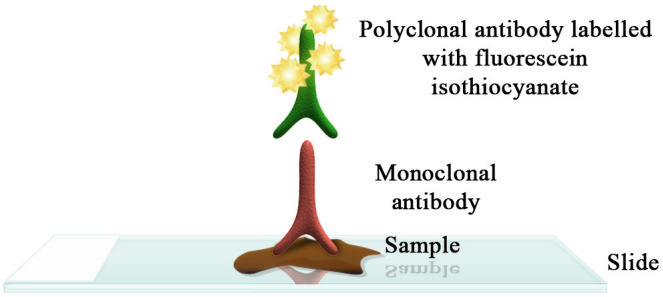
SECTION OR CELL CULTURE

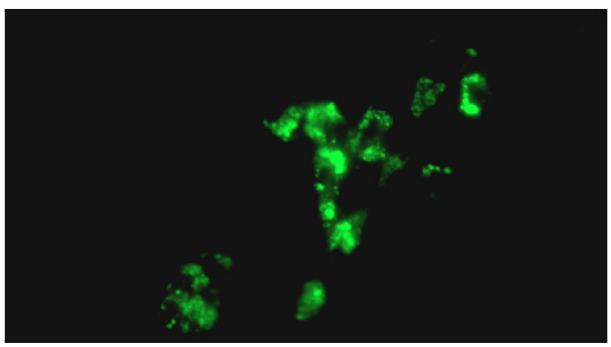
INTRODUCTION

Lawsonia intracellularis is an obligate intracellular bacterium causing proliferative enteropathy. The disease is characterised by adenomatous proliferation of epithelial cells infected by the bacterium and groosly seen as a thickening of intestinal mucosa. Diagnosis of *L. Intracellularis* infection in pigs is achieved by PCR of fecal or intestinal samples, indirect immunofluorescence antibody test (IFAT) of serum, as well as by in situ hybridisation, histology and immunohistochemical examination of formalin fixed samples of intestines for presence of intracellular bacteria in Veterinary microbiology epithelial cells.
BIO 323 recognises a 21 KD molecule in a western blotting analysis. This antigen is resistant to proteinase K digestion, suggesting it to be non-protein.
BIO 323 was tested highly specific for *L. intracellularis* by applying in situ hybridisation with a *L. intracellularis* specific probe targeting 16S ribosomal RNA simultaneously with the IFAT

Development, Characterization and diagnostic application of a monoclonal antibody specific for a proteinase K resistant Lawsonia intracellularis antigen.
Henriette T. Boesen, Tiom K. Jensen, Gregers Jungersen, Ulla Riber, Mette Boye, Kristian Moeller
Veterinary microbiology 105 (2005) 199-206

EXAMPLE OF RESULTS





Bio-X Diagnostics - 38, Rue de la Calestienne (PAE) - 5580 Rochefort - Belgique
Tél : 0032(0)84.32.23.77 - Fax : 0032(0)84.31.52.63 - E-mail : a.ginter@biox.com

BIO 323 could be used to perform direct immunofluorescence assay on frozen section or on deparaffined tissue section.

I – INDIRECT IMMUNOFLUORESCENCE ASSAY PROCEDURE ON FROZEN SECTION

Fix the tissue sections for 15 minutes at room temperature using 2% paraformaldehyde in PBS

Then rinse with PBS.
Dilute the reagent twentyfold in a PBS-Evans Blue solution prepared according to the following formula:

PBS-Evans Blue

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

Incubate the preparation on the sample for 1 hour at room temperature, preferably in a humidity chamber.
Upon completion of this incubation period rinse the preparation with a PBS solution.
Then add the conjugate (fluorescein-labelled anti-mouse immunoglobulin) at the manufacturer's recommended dilution. The conjugate available from Bio-X Diagnostics (Bio 305) should be diluted twentyfold in PBS-Evans Blue solution.
Incubate the preparation on the sample for 1 hour at room temperature, preferably, in a humidity chamber.
After this second incubation step rinse the preparation with PBS.

Dry the slide, then add the mounting medium made up as follows:

Mounting medium

Glycerol	9 parts by volume
PBS	1 part by volume

Place a cover slip on the slide, then observe under a microscope fitted for fluorescence detection.

The antibody may be kept in its original vial at 4°C for more than a year. Never freeze this reagent. Once diluted in the PBS-Evans Blue solution, the antibody remains stable for one week at 4°C.

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II – INDIRECT IMMUNOPEROXIDASE ASSAY PROCEDURE ON FROZEN SECTION

Fix the cell preparation (cell culture or tissue sections) for 15 minutes at room temperature using 2% paraformaldehyde in PBS

Then rinse with PBS.
Dilute the reagent twentyfold in PBS prepared according to the following formula:

PBS

NaCl:	8 gm
KH2PO4:	0.2 gm
KCl:	0.2 gm
Na2HPO4 . 2H2O:	1.15 gm
NaN3:	0.1 gm
H20	1 L

Incubate the preparation on the sample for 1 hour at room temperature, preferably in a humidity chamber.
Upon completion of this incubation period rinse the preparation with PBS.
Then add the conjugate (peroxidase-coupled anti-mouse immunoglobulin) at the manufacturer's recommended dilution. The conjugate available from Bio-X Diagnostics (Bio 269) should be diluted fiftyfold in PBS.
Incubate the preparation on the sample for 1 hour at room temperature, preferably in a humidity chamber.
After this second incubation step rinse the preparation with PBS.
Then add the chromogen (AEC, precipitating TMB, DAB, etc.) and the substrate (hydrogen peroxide) according to the manufacturer's instructions. Examine under the microscope for the presence of the coloured marker.

III – INDIRECT IMMUNOFLUORESCENCE OR IMMUNOPEROXIDASE ASSAY PROCEDURE ON DEPARAFFINED TISSUE SECTIONS

Tissue is placed in 10 % neutral-buffered formalin for 10 minutes After fixation, the material is dehydrated, embedded in paraffin, sectioned at 3 mm according to the normal procedure.
Slides are then deparaffinised before adding the monoclonal antibody.

Briefly:

Xylene 100 %	2 X 5 minutes
Ethanol 100 %	2 X 2 minutes
Ethanol 95 %	1 X 2 minutes
Ethanol 70 %	1 X 2 minutes

Water
Proteinase (P8038 Sigma 50 mgr/ml in TBS) (50 mM Tris, 150 mM NaCl, pH 7.6)

For immunoperoxidase labelling, the section is treated with 0.6 % H2O2 in TBS for 20 min in order to inhibit endogenous peroxidases. This step is not necessary for indirect immunofluorescence.
Rince the section with PBS then follow procedures described for tests on frozen sections (section I or II).

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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