# Bio-X Diagnostics

## ANTI-*LAWSONIA INTRACELLULARIS* MONOCLONAL ANTIBODY (BIO 323)

**(Reagent for indirect immunofluorescence or immunoperoxidase assay)**

**REAGENT FOR DETECTING *LAWSONIA INTRACELLULARIS* IN TISSUE SECTIONS.**

### I – INDIRECT IMMUNOFLUORESCENCE ASSAY PROCEDURE ON FROZEN SECTION

Fix the tissue sections for 15 minutes at 21°C +/- 3°C using 2% paraformaldehyde in PBS
Then rinse with PBS.
Dilute the reagent twentyfold in PBS prepared according to the following formula:

Phosphate Buffer Saline (PBS)

| | |
|---|---|
| NaCl: | 8 gm |
| $KH_2PO_4$: | 0.2 gm |
| KCl: | 0.2 gm |
| $Na_2HPO_4 . 2H_2O$: | 1.15 gm |
| $NaN_3$: | 0.1 gm |
| $H_20$ | 1 L |

Incubate the preparation on the sample for 1 hour at 21°C +/- 3°C, 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 156) should be diluted twentyfold in PBS-Evans Blue solution.

PBS-Evan Blue

| | |
|---|---|
| PBS | 1 liter |
| Evans Blue: | 0.01 gm |

Incubate the preparation on the sample for 1 hour at 21°C +/- 3°C, 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 between +2°C and +8°C for more than a year.
Never freeze this reagent.
Once diluted in PBS, the antibody remains stable for one week between +2°C and +8°C.

### II – INDIRECT IMMUNOPEROXIDASE ASSAY PROCEDURE ON FROZEN SECTION

Fix the cell preparation (cell culture or tissue sections) for 15 minutes at 21°C +/- 3°C 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 |
| $KH_2PO_4$: | 0.2 gm |
| KCl: | 0.2 gm |
| $Na_2HPO_4 . 2H_2O$: | 1.15 gm |
| $NaN_3$: | 0.1 gm |
| $H_20$ | 1 L |

Incubate the preparation on the sample for 1 hour at 21°C +/- 3°C, 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 157) should be diluted twentyfold in PBS.
Incubate the preparation on the sample for 1 hour at 21°C +/- 3°C, 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 0,07 % in TBS) (50 mM Tris, 150 mM NaCl, pH 7.6)

For immunoperoxidase labelling, the section is treated with 0.6 % $H_2O_2$ 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).