



LRM System Direct Immunofluorescence

REF 014-2013
Wave Direct Immunofluorescence Detection Kit
200 Tests

INTENDED USE

IVD For in vitro diagnostic use.

The Wave LRM (Linear Reagent Magazine) system is intended for laboratory use to detect antibodies bound to antigens on test tissues using direct immunofluorescence. This LRM may be used with either cells, frozen or formalin-fixed paraffin embedded tissues.

Positive results aid in the classification of normal and abnormal cells/tissues and serve as an adjunct to conventional histopathology. The clinical interpretation of any positive staining or its absence should be complemented by morphological and histological studies with proper controls. Evaluations should be made by a qualified individual in conjunction with the patient's clinical history and other diagnostic tests.

Refer to the Wave Instrument Operator's Manual for additional information concerning Materials Required but Not Provided; Storage; Staining Procedure; Troubleshooting; Interpretation of Staining; and General Limitations.

SUMMARY AND EXPLANATION

Immunofluorescence (IF) uses antibodies in conjunction with fluorescent dyes. This method is often utilized to visualize the cellular and subcellular distribution of biomolecules of interest. Labeled cells/tissues are visualized using a fluorescence microscope. In the Direct IF technique the staining procedure utilizes primary antibodies that are directly conjugated to fluorescent dyes.

This LRM system contains the necessary reagents for completing the sequential steps of Direct IF following application of the primary antibody. The user must provide the fluorescently labeled primary antibody, appropriately diluted in a Celerus User Fillable Primary Antibody Cartridge (PAC; Celerus Part No. 014-2502). When used together on the Wave instrument the PAC and the Direct IF LRM comprise a complete Direct IF test system.

PRINCIPLE OF PROCEDURE

Immunofluorescence is a multi-step process to identify specific cell markers within cells, tissue biopsies, or tumor specimens. The sequential steps may include antigen retrieval (optional), application of the fluorescently labeled primary antibody, followed by rinsing, and coverslipping the slide. Finished slides are then observed under fluorescence microscopy by trained personnel. The user must be familiar with the proper use of the fluorescence microscope including the selection of appropriate filters for use with the specific fluorescent dye. For further information on the staining procedure, refer to the Celerus Wave Operator's Manual.

MATERIALS PROVIDED

The LRM contains sufficient wash solution to process 200 slides. The system does not allow for reuse or refilling after the 200 slides have been stained.

MATERIALS REQUIRED BUT NOT PROVIDE

1. User Fillable PAC containing fluorescently-labeled primary antibody
2. Wave instrument
3. Wave slide rack
4. Positively-charged microscope slides, appropriately labeled
5. Timer
6. Celerus Riptide for antigen retrieval (or equivalent)
7. Celerus Target Retrieval Solution (or equivalent)
8. Slide drying chamber
9. Xylene or xylene substitute
10. Reagent alcohol or ethyl alcohol
11. Distilled or deionized water
12. TBS wash buffer, pH 7.6
13. Positive and negative tissue controls
14. Celerus Negative Control Reagent (or equivalent)
15. Mounting Medium
16. Cover slips

STORAGE AND HANDLING

Store LRM at 2-8 °C. Do not freeze. The reagents are stable up to the expiration date on the container. Do not use LRM after the expiration date, as the activity cannot be ensured.

There are no signs to indicate instability of these reagents. To ensure a valid staining assay, the use of positive and negative tissue controls is recommended. Contact your Celerus representative if there are stability concerns prior to the expiration date.

PRECAUTIONS

1. For professional users.
2. Minimize microbial contamination of reagents or an increase in nonspecific staining may occur.
3. As with any product derived from biological sources, proper handling procedures should be used.
4. A Material Safety Data Sheet is available for professional users on request.
5. ProClin 300 and sodium azide (NaN₃), used for stabilization, are not considered toxic in the concentrations used. ProClin 300 is classified per applicable European Community (EC) Directives as: Irritant (Xi). Sodium azide deposits in drainage pipes made of lead or copper can result in the formation of highly explosive metallic azides. To avoid such deposits in drainage pipes, sodium azide should be discarded in a large volume of running water. The following are the appropriate Risk (R) and Safety (S) phrases.



R22 Harmful if swallowed

R36 Irritating to eyes

R43 May cause sensitization by skin contact

R68 Possible risk of irreversible effects

S24 Avoid contact with skin

S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice

S37/39 Wear suitable gloves and eye/face protection

S46 If swallowed seek medical advice immediately and show this container or label

WASTE DISPOSAL

Adhere to all local laws when disposing of the LRM.

PACKAGING DAMAGE

DO NOT USE an LRM if it is leaking, has leaked, has spilled, cannot be primed, or has visually apparent physical damage.

SPECIMEN COLLECTION AND HANDLING

Formalin-fixed paraffin embedded (FFPE) tissues, frozen tissues, or smears are suitable for use. Wave detection kits have been optimized for tissues fixed with 10% formalin. Ideally, each 4-6µ tissue section should be placed on charged slides on the lower 2/3 of the slide. Very large sections should be placed 1/4 inch below the lower end of the slide label.

Slides should be baked overnight at 37 °C, or at 60 °C for one hour.

Use standard histochemical techniques to deparaffinize processed slides. For uniformity of staining results, it is recommended that target retrieval be performed using the Celerus Riptide and Celerus Target Retrieval Solution (or equivalent) at 112 °C for 5 minutes. Avoid drying of the tissue specimen during this process. After all slides to be stained have been inserted and reagents mounted on the instrument, start the staining run.

When the slides have completed the staining run, remove them from the instrument, cover slip, and view under light microscopy.

MOUNTING FINISHED SLIDES

When the slides have completed the staining run, remove them from the instrument and rinse 2 times in water. Mount finished slides in an aqueous mounting medium appropriate for use with immunofluorescence.

QUALITY CONTROL

Each staining run should include a known positive control specimen to ascertain a proper performance of all the applied reagents. If the positive control specimen fails to demonstrate positive results, staining of test specimens should be considered invalid. A negative control reagent should be used with each specimen to identify any non-specific staining.

If non-specific staining cannot be clearly differentiated from the specific staining, the staining of the test specimen should be considered invalid.

RESULTS EXPECTED

The LRM detection system, in conjunction with a fluorescently-labeled primary antibody, produces a fluorescent stain of antigen-antibody complexes. The specific color will vary, depending on the selection of the fluorescent dye. Positive staining should be present on the positive control specimen at the expected localization of the target antigen. If non-specific staining is present, this will be recognized as diffuse staining on the slides treated with the negative control reagent. Any non-specific staining or overstaining may require repeat testing. If retesting is required, follow the staining procedure outlined in the Celerus Wave Operator's Manual, with special care taken for deparaffinization and antigen retrieval. Over or under-fixed tissue may give erroneous results.



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