

**MultiVision Polymer Detection System:
MultiVision anti-rabbit/AP + anti-mouse/HRP polymers**

INTENDED USE:

For in vitro diagnostic use

AVAILABILITY:

Catalog #

Slide Volume

TL-012-MHRA

60-120 slides

SPECIFICITY:

Anti-Mouse IgG (H+L) and Anti-Rabbit IgG (H+L)

ENZYMES:

Peroxidase and Phosphatase

CHROMOGEN/ SUBSTRATE:

LVBBlue/ LVRed

REAGENTS:

| Qty | Component | TL-012-MHRA |
|-----|--|-------------|
| 1 | MultiVision anti-rabbit/AP + anti-mouse/HRP polymer (cocktail) | TL-012-RAM |
| 1 | Ultra V block | TA-012-UB |
| 1 | Hydrogen Peroxidase Block | TA-012-HP |
| 1 | LVBBlue AP Buffer | TA-075-TMM |
| 1 | LVBBlue Chromogen Vial 1 | TA-001-AB1 |
| 1 | LVBBlue Chromogen Vial 2 | TA-001-AB2 |
| 1 | LVBBlue Chromogen Vial 3 | TA-001-AB3 |
| 1 | LVRed Chromogen Vial 1 | TA-004-HR1 |
| 1 | LVRed Chromogen Vial 2 | TA-002-HR2 |
| 1 | LVRed Chromogen Vial 3 | TA-002-HR3 |
| 1 | LVRed Chromogen Vial 4 | TA-002-HR4 |
| 1 | Mounting Media | TA-005-UA |

SUPPLIED AS:

Ready-to-use polymer cocktail consisting of MultiVision anti-rabbit/AP + anti-mouse/HRP polymers.

LVBBlue for Alkaline Phosphatase activity and LVRed for Horseradish peroxidase activity.

DESCRIPTION

MultiVision Polymer Detection System is a simple and robust one-step polymer detection system for visualization of two antigens simultaneously in blue and red, respectively. A primary antibody cocktail of choice may be used in conjunction with ready-to-use MultiVision polymer cocktail, composed of anti-mouse/ horseradish peroxidase (HRP) + anti-rabbit/alkaline phosphatase (AP). This cocktail is specially formulated from MultiVision polymers that provide increased sensitivity, time-savings, and detection simplicity. The MultiVision AP and HRP polymers are innovative, patented technologies consisting of smaller amino acid polymer subunits that minimize conflicts in binding the target protein. The smaller polymer allows for decreased binding conflicts resulting in more consistent staining and better signal amplification. Ultimately, this gives the user higher sensitivity and antibody efficiency as well as better signal-to-noise ratios. This system is biotin-free, eliminating background staining found with traditional biotin-based methods. The MultiVision Polymer Detection System yields high quality double staining in less than 2 hours on formalin-

fixed and paraffin-embedded tissue sections. For optimal interpretation of results, appropriate positive and negative controls must be included.

PRINCIPLE OF THE PROCEDURE:

This MultiVision Polymer Detection System may be employed with any primary antibody cocktail to visualize the presence of two proteins simultaneously. After the specific antibodies are bound to their respective antigens in tissue sections, the rabbit and mouse primary antibodies are located by a specially formulated cocktail of secondary antibody polymers: anti-mouse/ horseradish peroxidase (HRP) + anti-rabbit/alkaline phosphatase (AP). In general, the amino acid polymer is conjugated to AP or HRP enzymes and the Fab fragments of goat anti-rabbit or goat anti-mouse immunoglobulins. The polymer complex is then visualized with first the LVBlue chromogen for AP activity and after washing with the LVRed chromogen for HRP activity. A weak hematoxylin nuclear counterstain is not recommended.

WARNINGS & PRECAUTIONS:

Clinical cases should be evaluated within the context of the performance of appropriate controls. Inclusion of a negative control fixed and processed in the same manner as the patient specimen placed on every slide run in addition to the case tissue is strongly recommended. For the test to be considered valid, the negative control should be clean. In some instances, very faint staining may be observed and is deemed acceptable. In addition, it is recommended that a negative tissue control slide be included for every batch of samples processed and run on the Lab Vision Autostainer. This negative tissue control should be included to ensure that the other treatment procedures did not create false positive staining.

Refer to MSDS for material safety instructions.

STORAGE AND STABILITY:

All components are stable for up to 12 months when stored at 2-8°C. Store chromogens away from light when possible.

MICROBIOLOGICAL STATE:

Product(s) are not sterile

MATERIALS REQUIRED BUT NOT PROVIDED:

Tissue pretreatment reagents.

SPECIMEN & REAGENT PREPARATION:

Refer to Procedure

PROCEDURE (MANUAL USE):

STAINING PROTOCOL (kit components in bold)

NOTE: The appropriate controls, especially negative controls, must be included with every manual or automated slide run. The inclusion of negative controls will aid in accurate interpretation of the staining results and help in determining false positives. Refer to the warnings and precaution section for details.

1. Paraffin tissue sections: deparaffinize in xylene substitute and rehydrate in graded alcohols

2. Wash 2 times in TBST buffer: (TBS + 0.1% Tween20).
3. Perform tissue pretreatment: heat-induced epitope retrieval for 20 min at 98°C followed by cooling at RT for 20 minutes.
4. Wash 4 times with DI water.
5. Apply **Hydrogen Peroxidase Block** solution and incubate 10 minutes at room temperature.
6. Wash 4 times in TBST.
7. Apply **Ultra V Block** and incubate for 10 minutes at room temperature to block nonspecific background staining. **Note:** do not exceed 10 minutes or there may be a reduction of staining intensity.
8. Do not wash after **Ultra V Block**, but blot off the blocking solution and continue with next step.
9. Apply **primary antibody cocktail** and incubate for 30 minutes at room temperature.
10. Wash 4 times in TBST.
11. Incubate with **MultiVision Polymer Cocktail**: anti-mouse/ horseradish peroxidase (HRP) + anti-rabbit/ alkaline phosphatase (AP) for 30 minutes at room temperature.
12. Wash 4 times in TBST.
13. Prepare working solution of **LVBlue** immediately before use and use within 15 minutes of preparation or decreased sensitivity may result.
14. Add 1 drop of **LVBlue vial 1** to 2.5 ml of **AP-buffer** and mix well.
15. Add 1 drop of **LVBlue vial 2** to AP-buffer and mix well.
16. Add 1 drop of **LVBlue vial 3** to AP-buffer and mix well.
17. Apply solution to tissue section and incubate for 10 minutes.
18. Wash 4 times in TBST.
19. Prepare working solution of **LVRed** immediately before use and use within 15 minutes of preparation or decreased sensitivity may result.
20. Add 3 drop of **LVRed vial 1** to 5 ml of DI water and mix well.
21. Add 2 drop of **LVRed vial 2** to mixture and mix well.
22. Add 2 drop of **LVRed vial 3** to mixture and mix well.
23. Add 2 drop of **LVRed vial 4** to mixture and mix well.
24. Apply solution to tissue section and incubate for 10 minutes.
25. Wash 4 times in DI water.
26. Dry tissue section completely, preferably at a 60°C hot plate for 1hr.
27. Mount with xylene-substitute containing medium provided with the kit. **NOTE 1:** Use of aqueous mounting media will make the **LVRed** reaction product disappear in a few days. **NOTE 2:** Use of xylene-containing mounting medium may partly dissolve the **LVBlue** reaction product.

PROCEDURE: (FOR AUTOSTAINER)

NOTE: The appropriate controls, especially negative controls, must be included with every manual or automated slide run. The inclusion of negative controls will aid in accurate interpretation of the staining results and help in determining false positives. Refer to the warnings and precaution section for details.

1. Paraffin tissue sections: deparaffinize in xylene-substitute and rehydrate in graded alcohols
2. Wash 2 times in TBST buffer.

3. Perform tissue pretreatment: heat-induced epitope retrieval for 20 minutes at 98°C followed by cooling at RT for 20 minutes.
4. Wash 4 times with DI water.
5. Apply **Hydrogen Peroxidase Block** solution and incubate 10 minutes at room temperature.
6. Rinse with TBST.
7. Apply **Ultra V Block** and incubate for 10 minutes at room temperature to block nonspecific background staining. Note: do not exceed 10 minutes or there may be a reduction of staining intensity.
8. Apply **primary antibody cocktail** and incubate for 30 minutes at room temperature.
9. Rinse with TBST.
10. Incubate with **MultiVision Polymer Cocktail: anti-mouse/ horseradish peroxidase (HRP) + anti-rabbit/alkaline phosphatase (AP)** for 30 minutes at room temperature.
11. Rinse with TBST.
12. Prepare fresh working solution of **LVBlue** immediately before use (follow steps 14 –16, above). Apply and incubate for 10min using the “Substrate-Batch” feature.
13. Rinse 3 times; once with TBST, followed by DI water, and TBST respectively.
14. Prepare fresh working solution of **LVRed** immediately before use (follow steps 20 –23 above). Apply and incubate for 10min using the “Substrate-Batch” feature.
15. Rinse 4 times with DI water.
16. Dry tissue section completely, preferably at a 60°C hot plate for 1hr.
17. Mount with Xylene-substitute mounting medium provided in the kit. NOTE 1: Use of aqueous mounting media will make the **LVRed** reaction product disappear in a few days. NOTE 2: Use of xylene-containing mounting medium may partly dissolve the **LVBlue** reaction product.

RECOMMENDATIONS:

1. Make sure that **LVBlue** and **LVRed** solutions are used at room temperature; temperature lower than room temperature may result in decreased staining; while temperatures too high may result in increased staining with additional non-specific background.
2. Alkaline phosphatase activity is inhibited by phosphate ions. Do not use PBS for washing and diluting reagents.

LIMITATIONS:

1. The use of this kit is limited to formalin-fixed and paraffin embedded specimen.
2. **LVBlue** reaction product is less crisp than **LVRed** reaction product. This may obscure observation.
3. Endogenous alkaline phosphatase is generally absent in formalin-fixed and paraffin-embedded tissue specimen. Endogenous AP activity present in intestinal tissues is not inhibited by levamisole and needs treatment before immunostaining (Bulman AS and Heyderman E, J. Clin. Pathol. 34:1349, 1981).

REFERENCES FOR DOUBLE STAINING:

1. Immunoenzyme Multiple Staining Methods. CM van der Loos, Handbook no. 45, BIOS Scientific Publishers, Oxford, UK, 1999. ISBN: 1-85996-187-8 or Springer-Verlag, New York, ISBN: 0-387-91594-x.
2. Multiple staining in molecular morphology, CM van der Loos. In: Molecular morphology in human tissues (Eds. Hacker GW and Tubbs RR), CRC Press, Boca Raton, FL, 2005, pp. 27-63