

SPECIFICATION SHEET

UltraVision LP Detection System HRP Polymer & DAB Plus Chromogen

DESCRIPTION:

UltraVision LP is the latest technology in polymeric labeling. Polymer detection methods have been shown to provide increased sensitivity and detection simplicity. This second-generation polymer system is composed of smaller polymer subunits that minimize conflicts in binding the target protein. Decreased binding conflicts result in more consistent staining and better signal amplification.¹ Ultimately, this gives the user higher sensitivity and antibody efficiency.² With **UltraVision LP**, you use less antibody and obtain better signal-to-noise ratios. **UltraVision LP** is also biotin-free, which eliminates background staining found with traditional biotin-based detection methods.

SPECIFICITY: Anti-Mouse IgG (H+L), Anti-Rabbit IgG (H+L)
ENZYME: Peroxidase
CHROMOGEN/SUBSTRATE: Diaminobenzidine (DAB)

AVAILABILITY:	<u>Catalog #</u>	<u>Slide Volume</u>
	TL-012-HD	120-240 slides
	TL-015-HD	150-300 slides

STAINING PROTOCOL (kit components in bold):

1. Deparaffinize and rehydrate tissue section.
2. Wash 2 times in buffer.
3. If required, incubate tissue in digestive enzyme (or appropriate pretreatment).
4. Wash 4 times in buffer.
5. To reduce nonspecific background staining due to endogenous peroxidase, incubate slide in **Hydrogen Peroxide Block** for 10-15 minutes.
6. Wash 4 times in buffer.
7. Apply **Ultra V Block** and incubate for 5 minutes at room temperature to block nonspecific background staining. Note: Do not exceed 10 minutes or there may be a reduction in desired stain. (May be omitted if primary antibodies are diluted in buffers containing 5-10% normal goat serum.)
8. Wash (Optional).
9. Apply primary antibody and incubate according to manufacturer's recommended protocol.
10. Wash 4 times in buffer.
11. Apply **Primary Antibody Enhancer** and incubate for 20 min at room temperature.

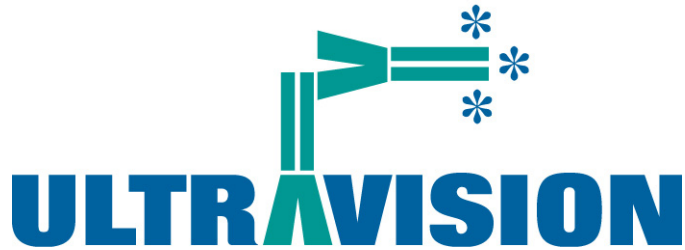
¹ Shan-Rong Shi, James Guo, Richard J. Cote, Lillian Young, Debra Hawes, Yan Shi, Sandra Thu, and Clive R. Taylor, Applied Immunohistochemistry & Molecular Morphology, vol 7, 201-208, 1999.

² Karen Petrosyan, Rosalba Tamayo, and Daisy Joseph, "Sensitivity of a Novel Biotin-free Detection Reagent (PowerVision+) for Immunohistochemistry" J. Histotechnology, vol 25, 247-250, 2002.



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12. Wash 4 times in buffer.
13. Apply **HRP Polymer** and incubate for 30 minutes at room temperature. (Note: HRP Polymer is light sensitive. Please avoid unnecessary light exposure and store in opaque vial.)
14. Wash 4 times in buffer.
15. Add 1-2 drops (40-100ul) **DAB Plus Chromogen** to 1 ml of **DAB Plus Substrate**, mix by swirling and apply to tissue. Incubate for 5-15 minutes, depending on the desired stain intensity.
16. Wash 4 times in DI water.
17. Counterstain and coverslip using a permanent mounting media.



PRECAUTIONS:

DAB Plus Chromogen: Contains Diaminobenzidine (DAB) in buffer. DAB is a suspected carcinogen. Do not pipet by mouth. Avoid contact with skin and eyes. Reagent is acidic and can cause burns if skin contact occurs. Handle with care and dispose of according to regulations.

DAB Plus Substrate: Contains Hydrogen Peroxide in buffer. Avoid contact with skin and eyes.

LIMITATIONS:

This product is available for laboratory use only - not for therapeutic work. LabVision Corporation will not be held responsible for patent infringement or other violation that may occur with the use of this product.

STORAGE: Store at 4-6°C. Each component is stable for 18 months.

TROUBLESHOOTING GUIDE

OVERSTAINING:

1. Concentration of the primary antibody was too high or the incubation time was too long.
2. Temperature during incubation was too high.
3. Incubation time with label was too long.

NONSPECIFIC BACKGROUND STAINING:

1. Rinsing between steps was inadequate.
2. Tissue was allowed to dry with reagents on.
3. Folds in tissue trapped reagents.
4. Tissue contains endogenous peroxidase.
5. Antigen migrated in tissue.
6. Excessive tissue adhesive on slides.
7. Inadequate blocking with protein block.

WEAK STAINING:

1. Primary antibody concentration was too low or incubation time was too short.
2. Reagents have degraded.
3. Inadequate removal of wash buffer between steps, resulting in dilution of reagents.
4. Counterstain or mounting media were incompatible and dissolved the chromogen reaction product.
5. Room temperature was excessively cool.



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6. The primary antibody does not recognize an antigen that survives fixation and embedding in high enough amounts.
7. Excessive incubation with protein block (Ultra Block or normal serum).

NO STAINING:

1. Steps were inadvertently left out.
2. There is no antigen in the tissue.
3. The primary antibody is not compatible with the detection method.
4. Chromogenic substrate has been replaced with another that is not intended for use with peroxidase.
5. One or more components of the kit have been inactivated.