VisUCyte™ HRP Polymer-DAB Cell & Tissue Staining Kit

For the detection of mouse, rabbit, or goat primary IgG antibodies with a biotin-free detection system

Size: 50 Tests

Secondary Antibody-HRP Polymer DAB System

Mouse Kit (Catalog Number VCTS001B)
Mouse/Rabbit (Catalog Number VCTS002B)
Rabbit Kit (Catalog Number VCTS003B)
Goat Kit (Catalog Number VCTS004B)

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

R&D Systems®' VisUCyte™ Cell and Tissue Staining Kits are intended for localization of antigens in a broad range of histological and cytological specimens. These kits may be used with primary IgG antibodies manufactured by R&D Systems or by other vendors. Detection is based on a secondary antibody and HRP enzyme conjugated to a polymer backbone for detection of primary antibodies that react with tissue antigens under study. Visualization is based on enzymatic conversion of a chromogenic substrate 3,3' Diaminobenzidine (DAB) into a colored brown precipitate by horseradish peroxidase (HRP) at the sites of antigen localization, which can then be viewed using bright- field microscopy.

Unlike earlier HRP detection systems, VisUCyte kits are biotin–free, eliminating the need to perform biotin and avidin blocking steps in stained samples (eliminates two blocking steps) and using a secondary antibody conjugated to a polymer backbone with a large number of HRP molecules. This one-step detection technique is 3-5 times more sensitive compared to conventional avidin-biotin protocols. VisUCyte Polymer detection reagents do not produce non-specific staining which may occur due to binding to phospholipids, nucleic acids and carbohydrate binding proteins.

R&D Systems' VisUCyte Cell and Tissue Staining Kits are manufactured to work equally well on cryostat and paraffin-embedded tissue sections, cytospin preparations (i.e., lymphocytes, monocytes, or transfected cells) and free-floating tissue sections. The kits are made in a ready-to-use format that eliminates the need for extra steps in the procedure, minimizes hands-on time, maximizes convenience, and reduces the risk of erroneous calculations.

LIMITATIONS OF THE PROCEDURE

- Do not interchange reagents of this kit with components from other R&D Systems' detection kits and/or similar components manufactured by other vendors.
- Not all primary antibodies are suitable for immunochemistry. Consult your vendor regarding application.
- Any variation in diluents, operator, pipetting technique, washing technique, and incubation time or temperature.
- Avoid dilution of reagents in this kit unless called for in the procedure.
- Reagents should be at room temperature before they are added to samples.

TECHNICAL HINTS

- DAB is not soluble in alcohol or xylene. Xylene-based mounting media may be used to mount tissues stained with DAB.
- Positive Control: To be sure that the staining system is working properly, include a specimen that is known to contain the antigen under study stained by other staining systems (*i.e.*, chromogenic, or fluorescent).
- Antibody Positive Control: To be sure that the primary antibodies will detect the antigen under study, include tissues that are known to contain that antigen (*i.e.*, if studying distribution of antigen X in liver that is known to be found in kidney, stain kidney tissue sections along with liver sections).
- Negative Control: This type of control is used to ensure that tissue staining is not caused by non-specific absorption of reagents to the tissue. Three types of negative control may be employed.
  1. **Null Control:** Substitute buffer for primary antibodies. The use of normal serum may not be considered a proper negative control because normal serum may contain unknown antibodies capable of binding to tissue non-specifically that will result in tissue staining.
  2. **Isotype Control:** When using monoclonal primary antibodies incubate control sample with non-immune immunoglobulin of the same isotype.
  3. **Absorption Control:** Incubate with antibodies that were pre-incubated with the corresponding immunogen. Absorption control works satisfactorily if immunogens are peptides. If antibodies were raised against proteins, addition of the mixture "antibodies plus proteins" to the tissue may result in higher non-specific staining that is most likely due to the formation of a sandwich complex "tissue-protein-antibody" that is recognized by secondary antibodies. Instead, absorption of antibodies by the immobilized protein may be employed.

PRECAUTIONS

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

3,3' Diaminobenzidine (DAB) is classified as a carcinogen. Wear gloves to avoid DAB contact with skin.

Follow local, state, and federal regulations to dispose of used DAB Chromogen.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse IgG Polymer HRP (Vial A)*	898521	6 mL of a anti-mouse secondary antibody in TBS, 1% (w/v) BSA, 0.1% Proclin 150, 10% heat-inactivated animal serum	Store at 2-8 °C until expiration date indicated on the kit components.
Rabbit IgG Polymer HRP (Vial A)*	898562	6 mL of a anti-rabbit secondary antibody in TBS, 1% (w/v) BSA, 0.1% Proclin 150, 10% heat-inactivated animal serum	
Mouse/Rabbit IgG Polymer HRP (Vial A)*	898522	6 mL of a anti-mouse/rabbit secondary antibody in TBS, 1% (w/v) BSA, 0.1% Proclin 150, 10% heat-inactivated animal serum	
Goat IgG Polymer HRP (Vial A)*	898563	6 mL of a anti-goat secondary antibody in TBS, 1% (w/v) BSA, 0.1% Proclin 150, 10% heat-inactivated animal serum	
DAB Chromogen	897076	1.5 mL of 2.5% 3,3' Diaminobenzidine (DAB) in stabilizing buffer.	
DAB Substrate Buffer	896076	3 vials (10 mL/vial) of a DAB stabilizing buffer.	
Horse Serum Blocking Buffer	897073	6 mL of animal serum in buffer with preservatives.	

*Vial (A) provided is dependent on kit ordered.

OTHER SUPPLIES REQUIRED

- Buffer (PBS, pH 7.4. Tris or Sodium Acetate-based buffers may be substituted)
- Mayer's Hematoxylin Counterstain (Sigma, Catalog # MHS16) or equivalent
- Non Aqueous Mounting Medium - Permount™ (Fisher Scientific™, Catalog # SP15-100) or equivalent
- Hydrogen Peroxide
- MeOH (Methanol)

STAINING PROCEDURE

Bring all reagents and samples to room temperature before use. Avoid drying of reagents added to samples by incubating them in a humidity chamber. If drying occurs, discard the specimens, and repeat the staining procedure using new samples.

1. Cover sample with a blocking reagent of choice depending on the tissue sample, MeOH or Peroxide for 5-15 minutes. The incubation time should be optimized.
   Note: *If the tissue is known to have endogenous peroxidase block with MeOH or hydrogen peroxide (ex. 30 % hydrogen peroxide in nine parts methanol for 10 minutes or 0.3–3.0 % hydrogen peroxide).*
   Note: *This procedure may affect morphology of unfixed frozen tissue sections. Reduction in incubation time may be required. This reagent has not been provided.*

2. Rinse sample with buffer and then wash in buffer for 5 minutes. Wash sample gently since hydrogen peroxide may loosen tissues from the slide.

3. Incubate sample with 50-150 µL of Horse Serum Blocking Buffer for 15 minutes at room temperature. Drain slides and carefully wipe off excess Horse Serum Blocking Buffer before going to the next step. Do not rinse with buffer.

4. Incubate sample with primary antibody. Follow manufacturer's recommendations regarding working dilution, time, and temperature of incubation.

5. Rinse sample with buffer. Wash three times in buffer for 5 minutes/wash.

6. Drain slides and carefully wipe off excess buffer before the next step.

7. Incubate sample with 50-150 µL of Secondary Antibody-Polymer HRP (Vial A) for 30-60 minutes at room temperature. Adjust the incubation time depending on the thickness of the section (the thicker the section, the longer the incubation time) and the affinity of primary antibodies.

8. Repeat Step 5.

9. Drain slides and carefully wipe off excess buffer before the next step.

10. Calculate the total volume of DAB Chromogen Solution needed for the entire reaction.
    Note: *100-200 µL of DAB Chromogen Solution is required to cover tissue section on a single slide. Add 30 µL of DAB Chromogen to 1 mL of DAB Chromogen Buffer.*

11. Mix DAB Chromogen Solution and the DAB Chromogen Buffer.

12. Add 50-150 µL of freshly prepared DAB Chromogen Solution to cover the entire sample and incubate for 3-10 minutes at room temperature. Monitor intensity of staining under a microscope to ensure proper intensity of tissue staining.
    Note: *DAB Chromogen Solution is stable for up to six hours.*

13. Rinse with distilled water and then wash in a fresh portion of distilled water for 5 minutes.
    Note: *DAB is not soluble in alcohol or xylene. Xylene-based mounting media (i.e., Per- mount) may be used to mount tissues stained with DAB.*

STAINING PROCEDURE *CONTINUED*

14. Samples stained with DAB may be either mounted without counterstaining or mounted after staining with hematoxylin.
    Note: *Excessive counterstaining with hematoxylin may mask areas with weak DAB staining.*
15. Place slides vertically on a filter paper or towel to drain excess mounting medium and let them dry.
16. Slides are ready for observation under the microscope.

SUGGESTED READINGS

1. A.E. Kalyuzhny (2016) *Immunohistochemistry: Essential Elements and Beyond* (Techniques in Life Science and Biomedicine for the Non-Expert); Springer International Publishing AG Part of Springer Nature.
2. Cuello, A.C. ed. (1993) *Immunohistochemistry: Methods in the Neurosciences*, Vol. 14; IBRO Handbook Series, John Wiley & Sons: New York.
3. Bullock, G.R. and P. Petrusz, eds. (1990) *Techniques in Immunocytochemistry*, Vol. 2; Academic Press: New York.
4. Lacey, A.J. ed. (1989) *Light Microscopy in Biology. A Practical Approach.* IRL Press: New York.
5. Bullock, G.R. and P. Petrusz, eds. (1989) *Techniques in Immunocytochemistry*, Vol. 4; Academic Press: New York.
6. Hsu, S.M. *et al.* (1981) Am. J. Clin. Pathol. **75**(5):734.
7. Hsu, S.M. *et al.* (1981) J. Histochem. Cytochem. **29**(4):577.

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