Cell & Tissue Staining Kit

For the detection of goat, mouse, rabbit, rat, or sheep primary IgG Antibodies

Size: 50 Tests

HRP-DAB System

Goat Kit (Catalog Number CTS008)
Mouse Kit (Catalog Number CTS002)
Rabbit Kit (Catalog Number CTS005)
Rat Kit (Catalog Number CTS017)
Sheep Kit (Catalog Number CTS019)

HRP-AEC System

Goat Kit (Catalog Number CTS009)
Mouse Kit (Catalog Number CTS003)
Rabbit Kit (Catalog Number CTS006)
Rat Kit (Catalog Number CTS018)
Sheep Kit (Catalog Number CTS020)

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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SECTION PAGE

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HRP-DAB SYSTEM

INTRODUCTION

R&D Systems' 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 the formation of the Avidin-Biotin Complex (ABC) with 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. The high detection sensitivity of R&D Systems' staining kits is achieved by using high quality secondary Biotinylated Antibodies and High Sensitivity Streptavidin conjugated to HRP (HSS-HRP). High Sensitivity Streptavidin is a chemical analog of Streptavidin that has little net positive charge at neutral or slightly alkaline pH and will interact only with biotin attached to secondary antibodies. HSS-HRP shows little or no non-specific binding to phospholipids, nucleic acids and carbohydrate binding proteins.

R&D Systems' 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 including pre-diluted secondary Biotinylated Antibodies and HSS-HRP 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, incubation time or temperature, and kit age can alter staining performance.
- 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. However, 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

Sodium azide (NaN_3) is added to the reagents as a preservative. NaN_3 may react with lead or copper plumbing to form explosive metal azides. Upon disposal, flush with a large volume of water to prevent azide build-up in drains. Due to NaN_3 toxicity, wear gloves to avoid contact with skin.

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. Please refer to the MSDS on our website prior to use.

The Biotinylated Secondary Antibodies in the mouse and rabbit Cell and Tissue Staining Kits (R&D Systems, Catalog # CTS002 and CTS005, respectively) contain human serum. Each donor used in the preparation of this material was tested by an FDA-approved method for the presence of antibody to human immunodeficiency virus (HIV) and hepatitis B surface antigen (HBsAg) and found to be negative. However, there can be no absolute assurance that HIV, HBsAg, or other infectious agents are absent. Consequently, this reagent should be handled as recommended for any potentially infectious material of human origin.

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
Peroxidase Blocking Reagent	865005	6 mL of 3% Hydrogen Peroxide (H_2O_2).	Store at 2-8 °C until expiration date indicated on the kit components.
Avidin Blocking Reagent	865009	6 mL of Avidin solution, containing 0.1% NaN_3.	
Biotin Blocking Reagent	865008	6 mL of Biotin solution, containing 0.1% NaN_3.	
Biotinylated Anti-mouse Antibody (Vial A)*	865001	6 mL of a anti-mouse secondary antibody in 0.01 M PBS containing 0.1% NaN_3.	
Biotinylated Anti-rabbit Antibody (Vial A)*	865002	6 mL of a anti-rabbit secondary antibody in 0.01 M PBS containing 0.1% NaN_3.	
Biotinylated Anti-goat Antibody (Vial A)*	865003	6 mL of a anti-goat secondary antibody in 0.01 M PBS containing 0.1% NaN_3.	
Biotinylated Anti-rat Antibody (Vial A)*	865113	6 mL of a anti-rat secondary antibody in 0.01 M PBS containing 0.1% NaN_3.	
Biotinylated Anti-sheep Antibody (Vial A)*	865114	6 mL of a anti-sheep secondary antibody in 0.01 M PBS containing 0.1% NaN_3.	
HSS-HRP (Vial B)	865006	6 mL of High Sensitivity Streptavidin conjugated to HRP in 0.01 M PBS containing 1% carrier protein, with preservatives and stabilizer.	
DAB Chromogen	860001	2 mL of 2.5% 3,3' Diaminobenzidine (DAB) in stabilizing buffer.	
DAB Chromogen Buffer	860005	2 vials (15 mL/vial) of 0.1% H_2O_2 in Tris HCl Buffer.	
Serum Blocking Reagent G	865004	6 mL of animal serum in buffer with preservatives. *Supplied in the Mouse CTS002, Rabbit CTS005, and Rat CTS017 kits.*	
Serum Blocking Reagent D	865015	6 mL of animal serum in buffer with preservatives. *Supplied in the Goat CTS008 and Sheep CTS019 kits.*	
Dropper Bottle	720180	15 mL empty dropper bottle.	

*Biotinylated vial 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 # MHS-16) or equivalent.
- Non Aqueous Mounting Medium - Permount™ (Fisher Scientific, Catalog # SP-15-100) or equivalent.

STAINING PROCEDURE

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 1-3 drops of the Peroxidase Blocking Reagent for 5 minutes.

   Note: *This procedure may affect morphology of unfixed frozen tissue sections. Reduction in incubation time may be required.*

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 1-3 drops of Serum Blocking Reagent G (for Mouse CTS002, Rabbit CTS005, and Rat CTS017 kits) or Serum Blocking Reagent D (for Goat CTS008 and Sheep CTS019 kits) for 15 minutes. Drain slides and carefully wipe off excess Serum Blocking Reagent before going to the next step. **Do not rinse with buffer.**

4. Incubate sample with 1-3 drops of Avidin Blocking Reagent for 15 minutes.

5. Rinse sample with buffer, drain slides and carefully wipe off excess buffer before the next step.

6. Incubate sample with 1-3 drops of Biotin Blocking Reagent for 15 minutes.

7. Rinse with buffer, drain slides and carefully wipe off excess buffer before the next step.

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

9. Rinse sample with buffer. Wash three times in buffer for 15 minutes/wash.

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

11. Incubate sample with 1-3 drops of Biotinylated Secondary Antibody (Vial A) for 30-60 minutes. Adjust the incubation time depending on the thickness of the section (the thicker the section, the longer the incubation time).

12. Repeat step 9.

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

14. Incubate sample with 1-3 drops of HSS-HRP (Vial B) for 30 minutes.

15. Rinse with buffer. Wash three times in buffer for 2 minutes/wash.

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

17. Calculate the total volume of DAB Chromogen needed for the entire reaction.
    Note: *100-200 µL of DAB Chromogen solution is required to cover tissue section on a single slide. Add two drops of DAB Chromogen to 2 mL of DAB Chromogen Buffer.*

STAINING PROCEDURE *CONTINUED*

18. Mix DAB Chromogen and the DAB Chromogen Buffer in the empty Dropper Bottle provided in the Kit.

19. Add 1-5 drops of freshly prepared DAB Chromogen solution from the Dropper Bottle to cover the entire sample and incubate for 3-20 minutes. 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.*

20. 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. Permount) may be used to mount tissues stained with DAB.*

21. 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.*

22. Place slides vertically on a filter paper or towel to drain excess mounting medium and let them dry.

23. Slides are ready for observation under the microscope.

HRP-AEC SYSTEM

INTRODUCTION

R&D Systems' 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 the formation of the Avidin-Biotin Complex (ABC) with primary antibodies that react with tissue antigens under study. Visualization is based on enzymatic conversion of a chromogenic substrate 3-amino-9-ethylcarbazole (AEC) into a colored red precipitate by horseradish peroxidase (HRP) at the sites of antigen localization, which can then be viewed using bright-field microscopy. The high detection sensitivity of R&D Systems' staining kits is achieved by using high quality secondary Biotinylated Antibodies and High Sensitivity Streptavidin conjugated to HRP (HSS-HRP). High Sensitivity Streptavidin is a chemical analog of Streptavidin that has little net positive charge at neutral or slightly alkaline pH and will interact only with biotin attached to secondary antibodies. HSS-HRP shows little or no non-specific binding to phospholipids, nucleic acids and carbohydrate binding proteins.

R&D Systems' 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 including pre-diluted secondary Biotinylated Antibodies and HSS-HRP 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

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- Not all primary antibodies are suitable for immunochemistry. Consult your vendor regarding application.
- Any variation in diluents, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can alter staining performance.
- 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.

TECHINCAL HINTS

- Since AEC is unstable in xylene, use only Aqueous Mounting Medium.
- 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).
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PRECAUTIONS

Sodium azide (NaN_3) is added to the reagents as a preservative. NaN_3 may react with lead or copper plumbing to form explosive metal azides. Upon disposal, flush with a large volume of water to prevent azide build-up in drains. Due to NaN_3 toxicity, wear gloves to avoid contact with skin.

AEC Chromogen is a flammable liquid and vapour. It causes serious eye irritation, is harmful in contact with skin or inhaled. It may damage the unborn child or may cause cancer. Wear gloves to avoid AEC contact with skin. Do not eat, drink, or smoke when using this product. Do not breathe fumes. Use only in a well-ventilated area. Keep away from heat, sparks, open flames, static and hot surfaces. Keep the container tightly closed. Refer to SDS for additional information or handling instructions. Follow local, state and federal regulations to dispose of used AEC Chromogen. Please refer to the MSDS on our website prior to use.

The Biotinylated Secondary Antibodies in the mouse and rabbit Cell and Tissue Staining Kits (R&D Systems, Catalog # CTS003 and CTS006, respectively) contain human serum. Each donor used in the preparation of this material was tested by an FDA-approved method for the presence of antibody to human immunodeficiency virus (HIV) and hepatitis B surface antigen (HBsAg) and found to be negative. However, there can be no absolute assurance that HIV, HBsAg, or other infectious agents are absent. Consequently, this reagent should be handled as recommended for any potentially infectious material of human origin.

MATERIALS PROVIDED & STORAGE CONDITIONS

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PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
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Biotin Blocking Reagent	865008	6 mL of Biotin solution, containing 0.1% NaN_3.	
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Biotinylated Anti-rabbit Antibody (Vial A)*	865002	6 mL of a anti-rabbit secondary antibody in 0.01 M PBS containing 0.1% NaN_3.	
Biotinylated Anti-goat Antibody (Vial A)*	865003	6 mL of a anti-goat secondary antibody in 0.01 M PBS containing 0.1% NaN_3.	
Biotinylated Anti-rat Antibody (Vial A)*	865113	6 mL of a anti-rat secondary antibody in 0.01 M PBS containing 0.1% NaN_3.	
Biotinylated Anti-sheep Antibody (Vial A)*	865114	6 mL of a anti-sheep secondary antibody in 0.01 M PBS containing 0.1% NaN_3.	
HSS-HRP (Vial B)	865006	6 mL of High Sensitivity Streptavidin conjugated to HRP in 0.01 M PBS containing 1% carrier protein, with preservatives and stabilizer.	
AEC Chromogen	860003	1 mL of 2% 3-amino-9-ethylcarbazole (AEC) in stabilizing buffer.	
AEC Chromogen Buffer	860004	2 vials (15 mL/vial) of buffer (< 10% methanol, DMSO, and water).	
Serum Blocking Reagent G	865004	6 mL of animal serum in buffer with preservatives. *Supplied in the Mouse CTS002, Rabbit CTS005, and Rat CTS017 kits.*	
Serum Blocking Reagent D	865015	6 mL of animal serum in buffer with preservatives. *Supplied in the Goat CTS008 and Sheep CTS019 kits.*	
Dropper Bottle	720180	15 mL empty dropper bottle.	

*Biotinylated vial 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 # MHS-16) or equivalent.
- Aqueous Mounting Medium: (R&D Systems, Catalog # CTS011).

STAINING PROCEDURE

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 1-3 drops of the Peroxidase Blocking Reagent for 5 minutes.

   Note: *This procedure may affect morphology of unfixed frozen tissue sections. Reduction in incubation time may be required.*

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 1-3 drops of Serum Blocking Reagent G (for Mouse CTS003, Rabbit CTS006, and Rat CTS018 kits) or Serum Blocking Reagent D (for Goat CTS009 and Sheep CTS020 kits) for 15 minutes. Drain slides and carefully wipe off excess Serum Blocking Reagent before going to the next step. **Do not rinse with buffer.**

4. Incubate sample with 1-3 drops of Avidin Blocking Reagent for 15 minutes.

5. Rinse sample with buffer, drain slides and carefully wipe off excess buffer before the next step.

6. Incubate sample with 1-3 drops of Biotin Blocking Reagent for 15 minutes.

7. Rinse with buffer, drain slides and carefully wipe off excess buffer before the next step.

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

9. Rinse sample with buffer. Wash three times in buffer for 15 minutes/wash.

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

11. Incubate sample with 1-3 drops of Biotinylated Secondary Antibody (Vial A) for 30-60 minutes. Adjust the incubation time depending on the thickness of the section (the thicker the section, the longer the incubation time).

12. Repeat step 9.

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

14. Incubate sample with 1-3 drops of HSS-HRP (Vial B) for 30 minutes.

15. Rinse with buffer. Wash three times in buffer for 2 minutes/wash.

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

17. Calculate the total volume of AEC Chromogen needed for the entire reaction.
    Note: *100-200 µL of AEC Chromogen solution is required to cover tissue section on a single slide. Add one drop of AEC Chromogen to 2 mL of AEC Chromogen Buffer.*

STAINING PROCEDURE *CONTINUED*

18. Mix AEC Chromogen and the AEC Chromogen Buffer in the empty Dropper Bottle provided in the Kit. **Note:** *AEC solution should be made immediately prior to use.*

19. Add 1-5 drops of freshly prepared AEC Chromogen solution from the Dropper Bottle to cover the entire sample and incubate for 3-20 minutes. Monitor intensity of staining under a microscope to ensure proper intensity of tissue staining.

20. Rinse with distilled water. Wash in fresh distilled water for 5 minutes.

21. Samples stained with AEC may be either mounted without counterstaining or mounted after staining with hematoxylin.
    Note: *Excessive counterstaining with hematoxylin may mask areas with weak AEC staining. Since AEC is unstable in xylene, use only Aqueous Mounting Medium.*

22. Place slides vertically on a filter paper or towel to drain excess mounting medium and let them dry.

23. Slides are ready for observation under the microscope.

SUGGESTED READINGS

1. Cuello, A.C. ed. (1993) *Immunohistochemistry: Methods in the Neurosciences*, Vol. 14; IBRO Handbook Series, John Wiley & Sons: New York.
2. Bullock, G.R. and P. Petrusz, eds. (1990) *Techniques in Immunocytochemistry*, Vol. 2; Academic Press: New York.
3. Lacey, A.J. ed. (1989) *Light Microscopy in Biology. A Practical Approach.* IRL Press: New York.
4. Bullock, G.R. and P. Petrusz, eds. (1989) *Techniques in Immunocytochemistry*, Vol. 4; Academic Press: New York.
5. Hsu, S.M. *et al.* (1981) Am. J. Clin. Pathol. **75**(5):734.
6. Hsu, S.M. *et al.* (1981) J. Histochem. Cytochem. **29**(4):577.