Immunohistochemistry

BETHYL LABORATORIES, INC

2-Step Immunoperoxidase Procedure: Formalin-fixed, Paraffin-embedded tissues and cell blocks

(3-5µm paraffin sections on charged (plus) slides)

Required Reagents:

Xylene
Ethanol- histology grade
Methanol
30% Hydrogen Peroxide
Hydrophobic barrier pen
Concentrated IHC Wash Solution (Bethyl cat# IHC-101e)
Ready-To-Use IHC Blocking Solution (Bethyl cat# IHC-101b)
Ready-To-Use IHC Antibody Diluent (Bethyl cat# IHC-101c)
Concentrated anti-Rabbit IHC Antibody (Bethyl cat# IHC-101d)
Concentrated DAB Substrate (Bethyl cat# IHC-101f)
Ready-To-Use IHC Hematoxylin (Bethyl cat# IHC-101g)
Ready-To-Use IHC Bluing Solution (Bethyl cat# IHC-101h)
Mounting media
Coverglass

Preparation of Reagents:

Methanol/H2O2
6 ml 30% Hydrogen Peroxide
194 ml Methanol
Prepare just prior to use.

Wash Solution
5 ml Concentrated IHC Wash Solution (cat# IHC-101e)
995 ml dH20
Store at 4 C, expiration 3 months

Secondary Antibody
1 drop Concentrated anti-Rabbit IHC Antibody (cat# IHC-101d)
2 ml Ready-To-Use Antibody Diluent (cat# IHC-101c)
Prepare just prior to use.

DAB Solution
2.5 ml dH20
Concentrated DAB Substrate (cat# IHC-101f):
1 drop DAB Solution A
1 drop DAB Solution B
1 drop DAB Solution C
Prepare just prior to use.

Procedure:

1. Xylene - 3 changes for 5 minutes each
2. 100% EtOH - 3 changes for 5 minutes each
3. Methanol/H2O2 - 5-15 minutes
4. dH20 rinse – 1 minute
5. Circle section with a hydrophobic barrier pen. Do not allow sections to dry for the remaining procedure.
6. Wash Solution - 3 changes for 5 minutes each
7. Ready-To-Use IHC Blocking Solution (cat# IHC-101b) - 15 minutes
8. Primary Antibody Incubation: 1 hour – room temperature. Prepare primary antibody with Ready-To-Use IHC Antibody Diluent (Bethyl cat# IHC-101c)
   Optimal working dilutions should be determined experimentally by the investigator. For Bethyl IHC Antibodies: 1:100 – 1:500
9. Wash Solution - 3 changes for 5 minutes each
10. Secondary Antibody Incubation: 1 hour – room temperature
11. Wash Solution - 3 changes for 5 minutes each
12. DAB Solution – 5-10 minutes (monitor development with microscope)
13. Wash Solution - 3 changes for 5 minutes each
14. Ready-To-Use IHC Hematoxylin (cat# IHC-101g) – 1-3 minutes
15. Wash Solution - 3 changes for 5 minutes each
16. Ready-To-Use IHC Bluing Solution (cat# IHC-101h) – 1-2 minutes
17. dH20 rinse
18. 70% EtOH – 3 minutes
19. 95% EtOH – 2 changes for 3 minutes each
20. 100% EtOH – 3 changes for 3 minutes each
21. Xylene - 3 changes for 3 minutes each
22. Mount and coverslip.
23. Place slides flat to dry.

2-Step Immunoperoxidase Procedure: Epitope Retrieval Method for Formalin-fixed, Paraffin-embedded tissues and cell blocks

(3-5µm paraffin sections on charged (plus) slides)

Required Reagents:

Xylene
Ethanol- histology grade
Methanol
30% Hydrogen Peroxide
Hydrophobic barrier pen
Concentrated Epitope Retrieval Buffer-Reduced pH (Bethyl cat# IHC-101a)
Concentrated IHC Wash Solution (Bethyl cat# IHC-101e)
Ready-To-Use IHC Blocking Solution (Bethyl cat# IHC-101b)
Ready-To-Use IHC Antibody Diluent (Bethyl cat# IHC-101c)
Concentrated anti-Rabbit IHC Antibody (Bethyl cat# IHC-101d)
Concentrated DAB Substrate (Bethyl cat# IHC-101f)
Ready-To-Use IHC Hematoxylin (Bethyl cat# IHC-101g)
Ready-To-Use IHC Bluing Solution (Bethyl cat# IHC-101h)
Mounting media
Coverglass

Preparation of Reagents:

Methanol/ H2O2
6 ml 30% Hydrogen Peroxide
194 ml Methanol
Prepare just prior to use.

Epitope Retrieval Buffer
4 ml Concentrated Epitope Retrieval Buffer-Reduced pH (Bethyl cat# IHC-101a)
196 ml dH20
Adjust pH 6.0 if necessary

Wash Solution
5 ml Concentrated IHC Wash Solution (cat# IHC-101e)
995 ml dH20
Store at 4 C, expiration 3 months

Secondary Antibody
1 drop Concentrated anti-Rabbit IHC Antibody (cat# IHC-101d)
2 ml Ready-To-Use Antibody Diluent (cat# IHC-101c)
Prepare just prior to use.

DAB Solution
2.5 ml dH20
Concentrated DAB Substrate (cat# IHC-101f):
1 drop DAB Solution A
1 drop DAB Solution B
1 drop DAB Solution C
Prepare just prior to use.

Procedure:

1. Xylene - 3 changes for 5 minutes each
2. 100% EtOH - 3 changes for 5 minutes each
3. Methanol/H2O2 - 5-15 minutes
4. dH20 rinse
5. Place prepared Epitope Retrieval Buffer into steamer or water bath and heat to 96 C- 100 C
6. Add rack of slides to hot Epitope Retrieval Buffer – 20 minutes
7. Remove container and slides from steamer or water bath and cool on bench – 20 minutes
8. Slowly add dH20 for 5 minutes
9. dH20 rinse – 1 minute
10. Circle section with a hydrophobic barrier pen. Do not allow sections to dry for the remaining procedure.
11. Wash Solution - 3 changes for 5 minutes each
12. Ready-To-Use IHC Blocking Solution (cat# IHC-101b) - 15 minutes
13. Primary Antibody Incubation: 1 hour – room temperature
    1. Prepare primary antibody with Ready-To-Use IHC Antibody Diluent (Bethyl cat# IHC-101c)
       Optimal working dilutions should be determined experimentally by the investigator.
       For Bethyl IHC Antibodies: 1:100 – 1:500
14. Wash Solution - 3 changes for 5 minutes each
15. Secondary Antibody Incubation: 1 hour – room temperature
16. Wash Solution - 3 changes for 5 minutes each
17. DAB Solution – 5-10 minutes (monitor development with microscope)
18. Wash Solution - 3 changes for 5 minutes each
19. Ready-To-Use IHC Hematoxylin (cat# IHC-101g) – 1-3 minutes
20. Wash Solution - 3 changes for 5 minutes each
21. Ready-To-Use IHC Bluing Solution (cat# IHC-101h) – 1-2 minutes
22. dH20 rinse
23. 70% EtOH – 3 minutes
24. 95% EtOH – 2 changes for 3 minutes each
25. 100% EtOH – 3 changes for 3 minutes each
26. Xylene - 3 changes for 3 minutes each
27. Mount and coverslip.
28. Place slides flat to dry.

2-Step Immunoperoxidase Protocol: Formaldehyde-Fixed Cells and Cytospin Preparations

Required Reagents:
3-4% Paraformaldehyde (freshly prepared) or 10% Neutral Buffered Formalin
Triton-X 100
Hydrophobic barrier pen
Concentrated IHC Wash Solution (Bethyl cat# IHC-101e)
Ready-To-Use IHC Blocking Solution (Bethyl cat# IHC-101b)
Ready-To-Use IHC Antibody Diluent (Bethyl cat# IHC-101c)
Concentrated anti-Rabbit IHC Antibody (Bethyl cat# IHC-101d)
Concentrated DAB Substrate (Bethyl cat# IHC-101f)
Ready-To-Use IHC Hematoxylin (Bethyl cat# IHC-101g)
Ready-To-Use IHC Bluing Solution (Bethyl cat# IHC-101h)
Mounting media
Coverglass

Preparation of Reagents:
Wash Solution
5 ml Concentrated IHC Wash Solution (cat# IHC-101e)
995 ml dH20
Store at 4 C, expiration 3 months

10% Triton-X 100 Stock
10 ml Triton-X 100
90 ml dH20

0.25% Triton-X 100
25 µl 10% Triton-X 100
1 ml TBS

Secondary Antibody
1 drop Concentrated anti-Rabbit IHC Antibody (cat# IHC-101d)
2 ml Ready-To-Use Antibody Diluent (cat# IHC-101c)
Prepare just prior to use.

DAB Solution
2.5 ml dH20
Concentrated DAB Substrate (cat# IHC-101f):
1 drop DAB Solution A
1 drop DAB Solution B
1 drop DAB Solution C
Prepare just prior to use.

Procedure:
1. For cells in chambered microscope slides or cells grown on coverslips: Gently rinse off media with TBS – 3 changes for 1 minute each. For cytospins: Allow to air dry after preparation.
2. Formaldehyde fixation – 10-30 minutes
3. Wash Solution - 3 changes for 5 minutes each
4. Circle cytospin with a hydrophobic barrier pen. Do not allow cells to dry for the remaining procedure.
5. 0.25% Triton-X 100 – 2-10 minutes - Optimal working dilutions and incubation times should be determined experimentally by the investigator.
6. Wash Solution - 3 changes for 5 minutes each
7. Ready-To-Use IHC Blocking Solution (cat# IHC-101b) - 15 minutes

8. Primary Antibody Incubation: 30 minutes – room temperature. Prepare primary antibody with Ready-To-Use IHC Antibody Diluent (Bethyl cat# IHC-101c). Optimal working dilutions should be determined experimentally by the investigator.
9. Wash Solution - 3 changes for 5 minutes each
10. Secondary Antibody Incubation: 30 minutes – room temperature.
11. Wash Solution - 3 changes for 5 minutes each
12. DAB Solution 5-10 minutes (monitor development with microscope)
13. Wash solution - 3 changes for 5 minutes each
14. Ready-To-Use IHC Hematoxylin (cat# IHC-101g) – 1-3 minutes
15. Wash Solution - 3 changes for 5 minutes each
16. Ready-To-Use IHC Bluing Solution (cat# IHC-101h) – 1-2 minutes
17. dH20 rinse
18. Remove chamber from microscope slide.
19. 70% EtOH – 3 minutes
20. 95% EtOH – 2 changes for 3 minutes each
21. 100% EtOH – 3 changes for 3 minutes each
22. Xylene - 3 changes for 3 minutes each
23. Mount and coverslip.
24. Place slides flat to dry.

NOTES:
This procedure is suitable for Phospho-specific Antibodies

2-Step Immunoperoxidase Protocol: Cells Grown in Culture and Cytospin Preparations

Required Reagents:
Acetone
Hydrophobic barrier pen
PBS
Ready-To-Use IHC Blocking Solution (Bethyl cat# IHC-101b)
Ready-To-Use IHC Antibody Diluent (Bethyl cat# IHC-101c)
Concentrated anti-Rabbit IHC Antibody (Bethyl cat# IHC-101d)
Concentrated DAB Substrate (Bethyl cat# IHC-101f)
Ready-To-Use IHC Hematoxylin (Bethyl cat# IHC-101g)
Ready-To-Use IHC Bluing Solution (Bethyl cat# IHC-101h)
Mounting media
Coverglass

Preparation of Reagents:
Secondary Antibody
1 drop Concentrated anti-Rabbit IHC Antibody (cat# IHC-101d)
2 ml Ready-To-Use Antibody Diluent (cat# IHC-101c)
Prepare just prior to use.

DAB Solution
2.5 ml dH20
Concentrated DAB Substrate (cat# IHC-101f):
1 drop DAB Solution A
1 drop DAB Solution B
1 drop DAB Solution C
Prepare just prior to use.

Procedure:

1. For cells in chambered microscope slides or cells grown on coverslips: Gently rinse off media with PBS 3 changes for 1 minute each. For cytospins: Allow to air dry after preparation.
2. Actone fixation (ice cold) 10 minutes
3. Air dry 30 minutes in hood
4. Circle cytospin with a hydrophobic barrier pen.
5. PBS-rinse. Do not allow sections to dry for the remaining procedure.
6. Ready-To-Use IHC Blocking Solution (cat# IHC-101b) - 15 minutes
7. Primary Antibody Incubation: 30 minutes room temperature . Prepare primary antibody with Ready-To-Use IHC Antibody Diluent (Bethyl cat# IHC-101c). Optimal working dilutions should be determined experimentally by the investigator.
8. PBS rinse- 3 changes for 5 minutes each
9. Secondary Antibody Incubation: 30 minutes room temperature
10. PBS rinse - 3 changes for 5 minutes each
11. DAB Solution 5-10 minutes (monitor development with microscope)
12. PBS rinse - 3 changes for 5 minutes each
13. Ready-To-Use IHC Hematoxylin (cat# IHC-101g) 1-3 minutes
14. dH20 rinse- 2 changes for 3 minutes each
15. Ready-To-Use IHC Bluing Solution (cat# IHC-101h) 1-2 minutes
16. dH20 rinse
17. Remove chamber from microscope slide.
18. 70% EtOH 3 minutes
19. 95% EtOH 2 changes for 3 minutes each
20. 100% EtOH 3 changes for 3 minutes each
21. Xylene - 3 changes for 3 minutes each
22. Mount and coverslip.
23. Place slides flat to dry.

2-Step Immunofluorescence Protocol: Formalin-fixed, Paraffin-embedded tissues and cell blocks

(3-5µm paraffin sections on charged (plus) slides)

Required Reagents:

Xylene
Ethanol- histology grade
Methanol
Hydrophobic barrier pen
Concentrated IHC Wash Solution (Bethyl cat# IHC-101e)
Ready-To-Use IHC Blocking Solution (Bethyl cat# IHC-101b)
Ready-To-Use IHC Antibody Diluent (Bethyl cat# IHC-101c)
Goat anti-Rabbit IgG Antibody-FITC (Bethyl cat# A120-201F)
Fluorescent mounting media
Coverglass

Preparation of Reagents:

Wash Solution
5 ml Concentrated IHC Wash Solution (cat# IHC-101e)
995 ml dH20
Store at 4 C, expiration 3 months

Secondary Antibody
20 µl Goat anti-Rabbit IgG Antibody-FITC (Bethyl cat# A120-201F)
2 ml Ready-To-Use Antibody Diluent (cat# IHC-101c)
Prepare just prior to use. Protect from light. Optimal working dilutions should be determined experimentally by the investigator.

Procedure:

1. Xylene - 3 changes for 5 minutes each
2. 100% EtOH - 3 changes for 5 minutes each
3. dH20 rinse 1 minute
4. Circle section with a hydrophobic barrier pen. Do not allow sections to dry for the remaining procedure.
5. Wash Solution rinse
6. Ready-To-Use IHC Blocking Solution (cat# IHC-101b) - 15 minutes
7. Primary Antibody Incubation: 1 hour room temperature. Prepare primary antibody with Ready-To-Use IHC Antibody Diluent (Bethyl cat# IHC-101c). Optimal working dilutions should be determined experimentally by the investigator. For Bethyl IHC Antibodies: 1:100 1:500
8. Wash Solution - 3 changes for 5 minutes each
9. Secondary Antibody Incubation: 1 hour room temperature. Protect from light.
10. Wash Solution - 3 changes for 5 minutes each
11. dH20 rinse
12. Mount with fluorescent mounting media and coverslip. Use fluorescent mounting media with DAPI if counterstaining is desired.

NOTES:
This procedure is suitable for Phospho-specific Antibodies

2-Step Immunofluorescence Protocol: Epitope Retrieval Method for Formalin-fixed, Paraffin-embedded tissues and cell blocks

(3-5µm paraffin sections on charged (plus) slides)

Required Reagents:

Xylene
Ethanol- histology grade
Methanol
Hydrophobic barrier pen
Concentrated Epitope Retrieval Buffer-Reduced pH (Bethyl cat# IHC-101a)
Concentrated IHC Wash Solution (Bethyl cat# IHC-101e)
Ready-To-Use IHC Blocking Solution (Bethyl cat# IHC-101b)
Ready-To-Use IHC Antibody Diluent (Bethyl cat# IHC-101c)
Goat anti-Rabbit IgG Antibody-FITC (Bethyl cat# A120-201F)
Fluorescent mounting media
Coverglass

Preparation of Reagents:

Epitope Retrieval Buffer
4 ml Concentrated Epitope Retrieval Buffer-Reduced pH (Bethyl cat# IHC-101a)
196 ml dH20
Adjust pH 6.0 if necessary
Wash Solution
5 ml Concentrated IHC Wash Solution (cat# IHC-101e)
995 ml dH20
Store at 4 C, expiration 3 months

Secondary Antibody
20 µl Goat anti-Rabbit IgG Antibody-FITC (Bethyl cat# A120-201F)
2 ml Ready-To-Use Antibody Diluent (cat# IHC-101c)
Prepare just prior to use. Protect from light. Optimal working dilutions should be determined experimentally by the investigator.

Procedure:

1. Xylene - 3 changes for 5 minutes each
2. 100% EtOH - 3 changes for 5 minutes each
3. dH20 rinse
4. Place prepared Epitope Retrieval Buffer into steamer or water bath and heat to 96 C- 100 C
5. Add rack of slides to hot Epitope Retrieval Buffer – 20 minutes
6. Remove container and slides from steamer or water bath and cool on bench – 20 minutes
7. Slowly add dH20 for 5 minutes
8. dH20 rinse – 1 minute
9. Circle section with a hydrophobic barrier pen. Do not allow sections to dry for the remaining procedure.
10. Wash Solution - 3 changes for 5 minutes each
11. Ready-To-Use IHC Blocking Solution (cat# IHC-101b) - 15 minutes
12. Primary Antibody Incubation: 1 hour – room temperature
    Prepare primary antibody with Ready-To-Use IHC Antibody Diluent (Bethyl cat# IHC-101c)
    Optimal working dilutions should be determined experimentally by the investigator.
    For Bethyl IHC Antibodies: 1:100 – 1:500
13. Wash Solution - 3 changes for 5 minutes each
14. Secondary Antibody Incubation: 1 hour – room temperature
15. Wash Solution - 3 changes for 5 minutes each
16. dH20 rinse
17. Mount with fluorescent mounting media and coverslip. Use fluorescent mounting media with DAPI if counterstaining is desired.

Notes:
This procedure is suitable for Phospho-specific Antibodies

2-Step Immunofluorescence Protocol: Formaldehyde-Fixed Cells and Cytospin Preparations

Required Reagents:
3-4% Paraformaldehyde (freshly prepared) or 10% Neutral Buffered Formalin
Triton-X 100
Hydrophobic barrier pen
Concentrated IHC Wash Solution (Bethyl cat# IHC-101e)
Ready-To-Use IHC Blocking Solution (Bethyl cat# IHC-101b)
Ready-To-Use IHC Antibody Diluent (Bethyl cat# IHC-101c)
Goat anti-Rabbit IgG Antibody-FITC (Bethyl cat# A120-101F)
Mounting media
Coverglass

Preparation of Reagents:
Wash Solution
5 ml Concentrated IHC Wash Solution (cat# IHC-101e)
995 ml dH20
Store at 4 C, expiration 3 months

10% Triton-X 100 Stock
10 ml Triton-X 100
90 ml dH20

0.25% Triton-X 100
25 µl 10% Triton-X 100
1 ml TBS

Secondary Antibody
20 µl Goat anti-Rabbit IgG Antibody-FITC (Bethyl cat# A120-101F)
2 ml Ready-To-Use Antibody Diluent (cat# IHC-101c)
Prepare just prior to use. Protect from light. Optimal working dilutions should be determined experimentally by the investigator.

Procedure:

1. For cells in chambered microscope slides or cells grown on coverslips: Gently rinse off media with TBS – 3 changes for 1 minute each. For cytospins: Allow to air dry for 1-2 minutes after preparation.
2. Formaldehyde fixation – 10-30 minutes
3. Wash Solution - 3 changes for 5 minutes each
4. Circle cytospin with a hydrophobic barrier pen. Do not allow cells to dry for the remaining procedure.
5. 0.25% Triton-X 100 – 2-10 minutes - Optimal working dilutions and incubation times should be determined experimentally by the investigator.
6. Wash Solution - 3 changes for 5 minutes each
7. Ready-To-Use IHC Blocking Solution (cat# IHC-101b) - 15 minutes
8. Primary Antibody Incubation: 30 minutes – room temperature. Prepare primary antibody with Ready-To-Use IHC Antibody Diluent (Bethyl cat# IHC-101c). Optimal working dilutions should be determined experimentally by the investigator.
9. Wash Solution - 3 changes for 5 minutes each
10. Secondary Antibody Incubation: 30 minutes – room temperature. Protect from light.
11. Wash Solution - 3 changes for 5 minutes each
12. dH20 rinse
13. Remove chamber from microscope slide.
14. Mount with fluorescent mounting media and coverslip. Use fluorescent mounting media with DAPI if counterstaining is desired.

NOTES:
This procedure is suitable for Phospho-specific Antibodies

2-Step Immunofluorescence Protocol: Fresh Frozen Tissue Sections
(5-15 µm cryosections on charged (plus) slides)

Required Reagents:
Acetone
Hydrophobic barrier pen
Concentrated IHC Wash Solution (Bethyl cat# IHC-101e)
Ready-To-Use IHC Blocking Solution (Bethyl cat# IHC-101b)
Ready-To-Use IHC Antibody Diluent (Bethyl cat# IHC-101c)
Goat anti-Rabbit IgG Antibody-FITC (Bethyl cat# A120-201F)
Mounting media
Coverglass

Preparation of Reagents:
Wash Solution
5 ml Concentrated IHC Wash Solution (cat# IHC-101e)
995 ml dH20
Store at 4 C, expiration 3 months

Secondary Antibody
20 µl Goat anti-Rabbit IgG Antibody-FITC (Bethyl cat# A120-201F)
2 ml Ready-To-Use Antibody Diluent (cat# IHC-101c)
Prepare just prior to use. Protect from light. Optimal working dilutions should be determined experimentally by the investigator.

Procedure:

1. Allow cryosections to air dry 30 minutes to 1 hour prior to fixation.
2. Actone (ice cold) 10 minutes
3. Air dry 30 minutes in hood
4. Circle section with a hydrophobic barrier pen.
5. Wash Solution - Do not allow sections to dry for the remaining procedure.
6. Ready-To-Use IHC Blocking Solution (cat# IHC-101b) - 15 minutes
7. Primary Antibody Incubation: 30 minutes room temperature. Prepare primary antibody with Ready-To-Use IHC Antibody Diluent (Bethyl cat# IHC-101c). Optimal working dilutions should be determined experimentally by the investigator.
8. Wash Solution - 3 changes for 5 minutes each
9. Secondary Antibody Incubation: 30 minutes room temperature. Protect from light.
10. Wash Solution - 3 changes for 5 minutes each
11. dH20 rinse
12. Mount with fluorescent mounting media and coverslip. Use fluorescent mounting media with DAPI if counterstaining is desired.

Immunohistochemistry Accessory Kit Protocol (Cat #: IHC-101)

Epitope Retrieval Method for Formalin-fixed, Paraffin-embedded tissues and cell blocks
(3-5µm paraffin sections on charged (plus) slides)

Required Reagents:
Immunohistochemistry Accessory Kit (Bethyl cat# IHC-101)
Kit contains:
Concentrated Epitope Retrieval Buffer-Reduced pH (Bethyl cat# IHC-101a)
Concentrated IHC Wash Solution (Bethyl cat# IHC-101e)
Ready-To-Use IHC Blocking Solution (Bethyl cat# IHC-101b)
Ready-To-Use IHC Antibody Diluent (Bethyl cat# IHC-101c)
Concentrated anti-Rabbit IHC Antibody (Bethyl cat# IHC-101d)
Concentrated DAB Substrate (Bethyl cat# IHC-101f)
Ready-To-Use IHC Hematoxylin (Bethyl cat# IHC-101g)
Ready-To-Use IHC Bluing Solution (Bethyl cat# IHC-101h)
Xylene
Ethanol- histology grade
Methanol
30% Hydrogen Peroxide
Hydrophobic barrier pen
Mounting media
Coverglass

Preparation of Reagents:
Methanol/ H2O2
6 ml 30% Hydrogen Peroxide
194 ml Methanol
Prepare just prior to use.

Epitope Retrieval Buffer
4 ml Concentrated Epitope Retrieval Buffer-Reduced pH (Bethyl cat# IHC-101a)
196 ml dH20
Adjust pH 6.0 if necessary

Wash Solution
5 ml Concentrated IHC Wash Solution (cat# IHC-101e)
995 ml dH20
Store at 40 C, expiration 3 months

Secondary Antibody
1 drop Concentrated anti-Rabbit IHC Antibody (cat# IHC-101d)
2 ml Ready-To-Use Antibody Diluent (cat# IHC-101c)
Prepare just prior to use.

DAB Solution
2.5 ml dH20
Concentrated DAB Substrate (cat# IHC-101f):
1 drop DAB Solution A
1 drop DAB Solution B
1 drop DAB Solution C
Prepare just prior to use.

Procedure:

1. Xylene - 3 changes for 5 minutes each
2. 100% EtOH - 3 changes for 5 minutes each
3. Methanol/H2O2 - 5-15 minutes
4. dH20 rinse
5. Place prepared Epitope Retrieval Buffer into steamer or water bath and heat to 960C- 1000C
6. Add rack of slides to hot Epitope Retrieval Buffer – 20 minutes
7. Remove container and slides from steamer or water bath and cool on bench – 20 minutes
8. Slowly add dH20 for 5 minutes
9. dH20 rinse – 1 minute
10. Circle section with a hydrophobic barrier pen. Do not allow sections to dry for the remaining procedure.
11. Wash Solution - 3 changes for 5 minutes each
12. Ready-To-Use IHC Blocking Solution (cat# IHC-101b) - 15 minutes
13. Primary Antibody Incubation: 1 hour – room temperature
    1. Prepare primary antibody with Ready-To-Use IHC Antibody Diluent (Bethyl cat# IHC-101c)
       Optimal working dilutions should be determined experimentally by the investigator.
       For Bethyl IHC Antibodies: 1:100 – 1:500
14. Wash Solution - 3 changes for 5 minutes each
15. Secondary Antibody Incubation: 1 hour – room temperature
16. Wash Solution - 3 changes for 5 minutes each
17. DAB Solution – 5-10 minutes (monitor development with microscope)
18. Wash Solution - 3 changes for 5 minutes each
19. Ready-To-Use IHC Hematoxylin (cat# IHC-101g) – 1-3 minutes
20. Wash Solution - 3 changes for 5 minutes each
21. Ready-To-Use IHC Bluing Solution (cat# IHC-101h) – 1-2 minutes
22. dH20 rinse
23. 70% EtOH – 3 minutes
24. 95% EtOH – 2 changes for 3 minutes each
25. 100% EtOH – 3 changes for 3 minutes each
26. Xylene - 3 changes for 3 minutes each
27. Mount and coverslip.
28. Place slides flat to dry.

Immunohistochemistry FAQs

What are the advantages of the IHC Accessory Kit?
Everything you need for immunostaining is included in the kit. The reagents are easy to prepare and use. The IHC Accessory kit processing times are shorter than the commonly used avidin-biotin systems. This kit does not utilize biotin in the system.

What is the cost per slide using the IHC Accessory Kit?
The kit will stain 250 slides at a cost of $1.26 per slide. The cost of the primary antibody is not included.

What is the cost per slide when using Bethyl IHC Antibodies?
Bethyl IHC antibodies average $2.50 per slide.

How does Bethyl qualify IHC Antibodies for FFPE?
All IHC Antibodies are tested on tissue microarrays using the IHC Accessory Kit.

Can I use the IHC Accessory Kit for phospho-specific antibodies?
Yes, the IHC Accessory Kit is suitable for phospho-specific antibodies.

Can I use Bethyl IHC antibodies for IF?
Yes, just substitute the anti-Rabbit IHC antibody with Bethyl's product A120-101F or A120-201F for the secondary detection. See procedure listed on this web site.

Are there advantages to using Immunoperoxidase (DAB) over Immunofluorescence (IF) for IHC?
Yes, with immunoperoxidase (DAB) methods you have a permanent slide representing your data. The surrounding tissue is visible to further enhance your data. Also, autofluorescence can confound your data.

When is it best to use IF?
IF is appropriate for Immunocytochemisty (ICC).

What positive control tissue should I use?
Refer to the individual IHC Antibody data sheet. All IHC Antibody data sheets have images demonstrating positive staining.

How long should FFPE tissues be fixed to be suitable for immunohistochemisty?
Formalin-fixed, paraffin-embedded tissues should be fixed in formaldehyde no longer than 24 hours. Tissues should not be thicker than 3mm to allow fixative penetration.