Product Manual

POLYVIEW® PLUS HRP-DAB (Anti-Rabbit) Kit

Catalog #: ENZ-KIT159-0150

150 Tests

For the latest product information, including support documentation, visit us online:
www.enzolifesciences.com

Technical Support (US): 800-942-0430
Technical Support (EU): +41 61 926 8989

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Product Manual

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Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

INTRODUCTION

The POLYVIEW® PLUS HRP-DAB (Anti-Rabbit) Kit provides reagents and materials for the ultrasensitive detection of rabbit antibodies used in both IHC and ISH staining. The kit is a non-biotin one-step detection system suitable for identifying antigens in formalin-fixed paraffin-embedded (FFPE) tissue samples and frozen sections. This kit may also be used with blood smears, cytosmears, and cell preparations.

POLYVIEW® PLUS HRP-DAB (Anti-Rabbit) Kit contains POLYVIEW® PLUS HRP (Anti-Rabbit) Reagent that has been developed by directly labeling anti-rabbit immunoglobulins with enzymes using a proprietary tandem hyper-labelling technology in a ready-to-use format. This ensures consistent and reproducible immunodetection of rabbit antibodies against nuclear, cytoplasmic and membrane antigens in different types of tissues and cells. The single step POLYVIEW® PLUS HRP (Anti-Rabbit) Reagent enables faster staining procedures compared to traditional two-step methods using biotin and avidin/streptavidin conjugates, with significantly lower background. Enzo's HIGHDEF® DAB Chromogen/Substrate and HIGHDEF® Hematoxylin reagents, coupled with the peroxidase and antibody blockers included in the kit, provide strong signal and give minimal background.

The POLYVIEW® PLUS HRP-DAB (Anti-Rabbit) Kit is suitable for use with all rabbit IgG antibodies, both monoclonal and polyclonal. The reagents from this kit can be used for manual staining or with automated staining instruments and is well suited for multiplex immunohistochemical staining assays.

MATERIALS SUPPLIED

IHC/ISH Peroxidase Block (Green Cap)	22.5 mL
Antibody Blocker/Diluent (Red Cap)	45 mL
POLYVIEW® PLUS HRP (Anti-Rabbit) Reagent (Orange Cap)	22.5 mL
Antigen Retrieval Reagent, pH 9 (10X)	100 mL
HIGHDEF® DAB Chromogen (Amber Vial)	1 mL
HIGHDEF® DAB Substrate Buffer (White Cap)	22.5 mL
HIGHDEF® Hematoxylin (Blue Cap)	22.5 mL


Storage temp

STORAGE

Store all of the kit components at 2°-8°C. Do not freeze. The HIGHDEF® Hematoxylin may be stored at room temperature away from direct bright light.

STABILITY

When used and stored as directed, the kit is stable until the expiration date indicated on the box.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

- PAP pen (Prod. No. ADI-950-233-0001)
- Proteinase K for ISH (Prod. No. ENZ-33801)
- DIGX® Rabbit anti-digoxigenin linker (Ready-to-Use) (Prod No. ENZ-ABS303-6000) (For use with digoxigenin labelled ISH probes)
- Rabbit anti-biotin linker (Ready-to-use) (Prod. No. ENZ-32892-6000) (For use with biotin labelled ISH probes)
- Slide holder
- Xylene (or Xylene substitute) for dewaxing
- 50%, 70%, 90% and 100% ethanol (Reagent Grade)
- Distilled or deionized water
- PBST Wash Buffer (0.05% Tween-20® in 1X PBS)
- Specimen slides and coverslips
- HIGHDEF® Mount (Prod. No. ADI-950-261-0030)
- ISH Wash Buffer (Prod. No. ENZ-33809)
- Heating oven set at ≥60°C
- Heating blocks for slides
- Absorbent wipes
- Timer
- Also available: Antigen Retrieval Reagent, pH 6 (Prod. No. ENZ-ACC112)

PRECAUTIONS

1. **For RESEARCH use only**. Not to be used for *in vitro* diagnostic purposes.
2. Wear appropriate personnel protective apparel. Avoid contact with clothes and exposed skin. In case of accidental skin exposure, flush with water immediately. Consult a physician if required.
3. Use a safety pipetting device for all pipetting. Never pipet by mouth.
4. Interpretation of the results is the sole responsibility of the user.

REAGENT PREPARATION

PBST Wash Buffer:

To prepare one liter of wash buffer, add 500 µL of Tween-20® to 1 liter of PBS.

DAB Chromogen/Substrate:

This reagent must be prepared shortly before use. Per 1 mL of HIGHDEF® DAB Substrate Buffer, add 40 µL of HIGHDEF® DAB Chromogen. Mix well. Keep protected from direct bright light. When refrigerated and protected from light, this solution is stable for up to 7 days.

1X Antigen Retrieval Solution:

To prepare 100 mL of 1X Antigen Retrieval Solution, add 10 mL of Antigen Retrieval Reagent (10X) to 90 mL of deionized water. Mix well and keep protected from bright light. For best results, use immediately.

Note: Also available is Antigen Retrieval Reagent, pH 6 (Prod. No. ADI-950-270). However, we recommend using this kit for with Antigen Retrieval Reagent, pH 9, as provided.

STAINING PROTOCOL – ISH (*IN SITU* HYBRIDIZATION)

NOTE: *(1) Do not allow the slides to dry between steps during the entire hybridization and detection procedures, or erroneous results will occur. Add sufficient amounts of reagents to specimens during incubation steps and cover the slides while incubating to avoid drying.*

(2) If weak signal is noticed, increase the incubation times for hybridization and detection steps.

I. SPECIMEN SLIDE PREPARATION AND PRETREATMENT

The POLYVIEW® PLUS HRP-DAB (Anti-Rabbit) kit can be used on formalin-fixed, paraffin embedded (FFPE) biopsy sections. It can also be used on fixed cells. No special preparative materials are required for use of the system on fixed cells although pretreatment of the slides is required (see section B).

A. BIOPSY SLIDES

Note: *Paraffin-embedded biopsy specimen slides must be deparaffinized and Proteinase K treated prior to* in situ *hybridization procedures.*

1. Apply one to three FFPE sections (4-6 microns thick) of each biopsy specimen to a specimen slide.

   Bake tissue-mounted slides vertically for at least 2 hr (up to 18 hr) at 60-80°C to fix the slides. Store fixed slides at room temperature.

2. Arrange the tissue specimen mounted slides in a slide holder.

   Note: *(1) If slides will be used immediately after sections are fixed on the slides by baking, proceed from step 4.*

   (2) If wax on slides has solidified upon storage, proceed from step 3.

3. Transfer the slides in drying oven at 55-60°C for 20 min. to melt the wax.

4. Deparaffinize the specimen slides by soaking them sequentially in the following solutions for the time indicated:

Soak Number	Reagent	Duration of Soak
1	Xylene (or Xylene substitute)	5 min
2	Xylene (or Xylene substitute)	5 min
3	100% Ethanol	3 min
4	100% Ethanol	3 min
5	90% Ethanol	1 min
6	70% Ethanol	1 min
7	50% Ethanol	1 min
8	Deionized Water	1 min

   Caution: *Xylene saturates rapidly with paraffin. Replace the solutions for each batch of slides.*

5. After the final soak, wipe the excess liquid around the section on the glass slide and encircle the tissue section with a PAP pen and allow to dry for 10 sec.

6. Transfer the slides to a 37°C warm plate.
7. Add the optimal concentration of Proteinase K (recommended 25 - 80 µg/mL, varies with tissue type and thickness) to cover the tissue section (~150 µL, 4 drops), cover the slides to prevent drying, and incubate for 10 min. on the 37°C warm plate.
8. Wash for 5 min. with PBST Wash Buffer.
9. Inactivate any endogenous peroxidase activity by incubating each specimen in enough IHC/ISH Peroxidase Block to cover the tissue section (~150 µL, 4 drops, the volumes should be enough to cover the tissue section, and not dry out during the incubation) for 8 min. at room temperature. Wash the slides with PBST (see Reagent Preparation section) for 2 min.
10. Dehydrate the specimen slides by incubating at room temperature in the following solutions for the times indicated:

Soak Number	Reagent	Duration of Soak
1	Deionized Water	1 min
2	50% Ethanol	1 min
3	75% Ethanol	1 min
4	100% Ethanol	1 min

11. Tap off excess ethanol, and air dry for 1 minute.
12. Proceed to the Hybridization of Probe section.

B. FIXED CELL SPECIMEN SLIDES

1. Place specimen slides containing fixed cells on the 37°C heating block (slide warmer) and add 0.5 to 0.7 mL of PBST Wash Buffer. Incubate slides at 37°C for 15 min. Tap off wash buffer. Dehydrate slides by incubating at room temperature in the following solutions for the times indicated:

Soak Number	Reagent	Duration of Soak
1	Deionized water	1 min
2	50% Alcohol	1 min
3	75% Alcohol	1 min
4	100% Alcohol	1 min

2. Proceed to the Hybridization of Probe section.

II. HYBRIDIZATION OF PROBE

1. Hybridize the probe as recommended by the probe manufacturer. Times and temperatures will vary by probe type. The investigator needs to optimize the concentrations and incubation times of hapten-labeled (*i.e.,* Biotin, Digoxigenin or Fluorescein) RNA or DNA probes (recommended 10 min denaturation at 95°C and >120 min for hybridization, typically 37°C for DNA probes or 42°C for RNA probes). A coverslip must be placed after adding the probe to the specimen.
2. Wash for 5 min with PBST Wash Buffer.
3. After hybridization, wash the slides with Hybridization Wash Buffer (see Reagent list) for 10 min., followed by a 5 min wash with PBST.

III. ISH DETECTION

1. Block each specimen slide in enough Antibody Blocker/Diluent to cover the tissue section (~150 µL, 6 drops) for 10 min. This will prevent the nonspecific binding of POLYVIEW® PLUS HRP (Anti-Rabbit) Reagent.
2. Wash the slides in PBST for 2 minutes. Tap off excess wash solution.
3. Add enough volume of an optimal concentration of rabbit anti-hapten (*i.e.,* Biotin, Digoxigenin or Fluorescein; not provided) linker to cover the specimen, and incubate for 15 min. at room temperature.

   NOTE: To reduce background, further dilution of Ready-To-Use Antibody Linker may be necessary.
4. Wash the slides twice in PBST for 2 min. each wash. Tap off excess wash solution.
5. To each specimen add POLYVIEW® PLUS HRP (Anti-Rabbit) Reagent to cover the tissue (~150 µL, 6 drops). Incubate for 20 min. at room temperature.
6. Wash the slides twice in PBST for 5 min each was. Tap off excess wash solution.
7. Add DAB Chromogen/Substrate (see Reagent Preparation section, ~150 µL, 4 drops) to specimen and allow color to develop for 5 min. at room temperature.
8. Tap off excess DAB Chromogen/Substrate and wash the slides twice with water. Tap off excess water.
9. Counterstain each specimen with Hematoxylin (~150 µL, 4 drops) for 5 min. at room temperature.

10. Tap off excess counterstain and rinse the slides with water.
11. Using water or HIGHDEF® Mount as mounting medium, view the slides using a light microscope.

HPV – ISH

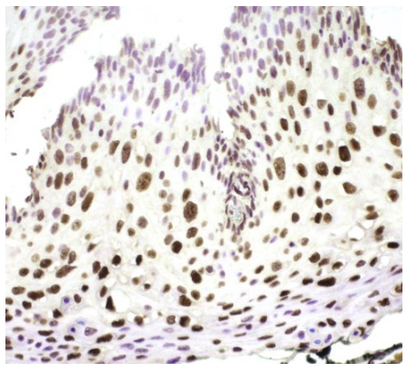

Figure 1. Enzo's Biotin-labeled High Risk HPV DNA probes and POLYVIEW® PLUS HRP-DAB (Anti-Rabbit) Kit used to perform ISH.

STAINING PROTOCOL – IHC (IMMUNOHISTOCHEMISTRY)

NOTE: *(1) Do not allow the slides to dry between steps during the entire procedure. Add sufficient amounts of reagents to specimens during incubation steps and cover the slides while incubating to avoid drying.*

(2) If weak signal is noticed, increase the incubation times for primary antibody and the POLYVIEW® PLUS HRP (Anti-Rabbit) Reagent.

I. SPECIMEN SLIDE PREPARATION AND PRETREATMENT

The POLYVIEW® PLUS HRP-DAB (Anti-Rabbit) kit can be used on formalin-fixed, paraffin embedded (FFPE) biopsy sections. It can also be used on fixed cells. No special preparative materials are required for use of the system on fixed cells although pretreatment of the slides is required (see section I-B of *in situ* Hybridization section).

A. BIOPSY SLIDES

Note: *Paraffin-embedded biopsy specimen slides must be deparaffinized and treated with an Antigen Retrieval Reagent prior to* in situ *hybridization procedures.*

1. Apply one to three FFPE sections (4-6 microns thick) of each biopsy specimen to a specimen slide.

   Bake tissue-mounted slides vertically for at least 2 hrs. (up to 18 hr.) at 60-80°C to fix the slides. Store fixed slides at room temperature.
2. Arrange the tissue specimen mounted slides in a slide holder.

Note: *(1) If slides will be used immediately after sections are fixed on the slides by baking, proceed from step 4.*

(2) If wax on slides has solidified upon storage, proceed from step 3.

3. Transfer the slides in drying oven at 55-60°C for 20 min to melt the wax.
4. Deparaffinize the specimen slides by soaking them sequentially in the following solutions for the time indicated:

Soak Number	Reagent	Duration of Soak
1	Xylene (or Xylene substitute)	5 min x 2
2	100% Ethanol	3 min x 2
3	90% Ethanol	1 min
4	70% Ethanol	1 min
5	50% Ethanol	1 min
6	Deionized Water	1 min

Caution: *Xylene saturates rapidly with paraffin. Replace the solutions for each batch of slides.*

5. After the final soak, wipe the excess liquid around the section on the glass slide and encircle the tissue section with a PAP pen. Dry for 10 sec.
6. Retrieve antigen with 1X Antigen Retrieval Solution (see Reagent Preparation section) for 20 min at 99° C.
7. Wash slides with PBST for 5 min.
8. Inactivate any endogenous peroxidase activity by incubating each specimen in enough IHC/ISH Peroxidase Block to cover the tissue section (~150 µL, 6 drops) for 8 min at room temperature.
9. Wash the slides with PBST for 5 min.

II. ANTIBODY BLOCKING AND PRIMARY ANTIBODY STAINING

Note: *If blocking is required before incubation with primary antibody, use the Antibody Blocker/Diluent provided in the kit. DO NOT use normal rabbit serum as blocking reagent.*

1. Block each specimen slide in enough Antibody Blocker/Diluent to cover the tissue section (~150 µL, 6 drops) for 10 min. This will prevent the nonspecific binding of POLYVIEW® PLUS HRP (Anti-Rabbit) Reagent.
2. Wash the slides twice in PBST for 5 min each wash. Tap off excess wash solution.
3. Add enough volume of an optimal concentration of primary rabbit antibody to cover the specimen, and incubate for 20-30 min at room temperature. Refer to the primary antibody manufacturer's recommendations for optimal incubation conditions and dilutions.
4. Wash the slides 3 times in PBST for 2 min. each. Tap off excess wash solution. Proceed to IHC Detection section.

III. IHC DETECTION

1. Block each specimen slide in enough Antibody Blocker/Diluent to cover the tissue section (~150 µL, 6 drops) for 10 min. This will prevent the nonspecific binding of POLYVIEW® PLUS HRP (Anti-Rabbit) Reagent.
2. Wash the slides twice in PBST for 5 min each wash. Tap off excess wash solution.
3. To each specimen add POLYVIEW® PLUS HRP (Anti-Rabbit) Reagent to cover the tissue (~150 µL, 6 drops). Incubate for 30 to 60 min. at room temperature.
4. Wash the slides three times in PBST for 2 min. each. Tap off excess wash solution.
5. Add DAB Chromogen/Substrate (see Reagent Preparation section) to cover tissue section (~150µL, 4 drops) and allow color to develop for 5 min. at room temperature.
6. Wash the slides with water. Tap off excess water.
7. Counterstain each specimen with HIGHDEF® Hematoxylin (~150 µL, 4 drops) for 1-2 min. at room temperature. Incubate longer, up to 5 minutes, as desired for darker counterstaining.
8. Tap off spent counterstain and rinse the slides with water.
9. Using water or other mounting medium, view the slides using a light microscope.

   If desired, dehydrate the slides in 70% alcohol for 1 min. followed by 100% alcohol for 1 min. Then, clear sections in xylenes for 1 min and then mount using permanent histological mounting medium.

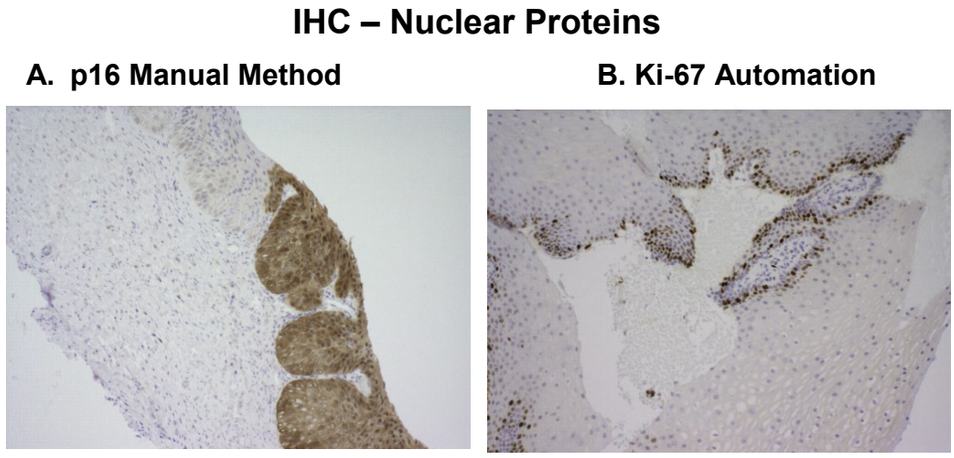

Fig 2. P16 clone E6H4, Ki-67 clone MIB-1 and POLYVIEW® PLUS HRP-DAB (Anti-Rabbit) Kit (Enzo Life Sciences) was used to perform IHC both manual method (**A**) and Biocare automation (**B**).

IV. OPTIONAL USE OF RABBIT LINKER WHEN USING MOUSE PRIMARY ANTIBODY

When using mouse primary antibody, Rabbit anti-Mouse Linker (available separately; ENZ-ACC116) is necessary to use with this kit. Prior to incubation with Rabbit anti-Mouse Linker, block the sections for 10 minutes with antibody blocking buffer. Refer to the product user's instructions for details on its use.

TROUBLESHOOTING GUIDE

Problem	Reason	Solution
No signal noticed on any slide	Wrong reagent used.	Carefully follow instructions as described in this manual. Do not substitute kit reagents.
	Sodium azide contamination	Use sodium azide free buffers
Weak signal	Primary antibody concentrations may be low.	Increase the primary antibody concentration and/or incubate it longer.
	Inefficient removal of excess wash solutions.	Gently tap off wash solutions after each wash. Wipe off excess washings around the specimen, if necessary.
	Incubations with the detection reagents may be too short.	Increase incubation times.
	Paraffin or pap pen covered the section	When paraffin is noticed on sections, expose slides to 100% ethanol for 5 min and wash with water. Try to encircle apart from the tissue with pap pen.
Moderate background	Sections dried during the procedure.	Process 4-5 slides at a time (when processing manually). Cover the slides during incubations to avoid rapid evaporation of reagents.
	Nonspecific binding of reagents to tissue	Increase incubation time with blocking reagent.
	Primary antibody used may be too concentrated.	Dilute the primary antibody further.
	Paraffin not removed completely.	Incubate section 5 more min in 100% ethanol followed by water. Replace de-waxing reagents more frequently.
High background	Slides are not properly washed.	Use PBST Wash Buffer as recommended in the procedures, if using a different wash buffer.
	Too harsh conditions for the Antigen Retrieval Reagent	Use a different antigen retrieval method, or reduce time or temperature.
	Substrate incubation is too long.	Shorten the incubation time in DAB Chromogen/Substrate.
	Antibody concentration is too high.	Dilute the primary antibody or linker concentration.
	High level of endogenous peroxidase.	Increase the peroxidase block incubation from 5 min to 10 min.
	Tissue has high levels of biotin (if detecting Biotin probes).	Use avidin biotin blocking.
Tissue falling off slide	Antigen retrieval is too harsh.	Use a different antigen retrieval method, or reduce time or temperature.
	Proteinase K, if used, is too strong.	Reduce or eliminate the amount of proteinase K used.

NOTES

GLOBAL HEADQUARTERS
Enzo Life Sciences Inc.
10 Executive Boulevard
Farmingdale, NY 11735
Toll-Free:1.800.942.0430
Phone:631.694.7070
Fax: 631.694.7501
info-usa@enzolifesciences.com

EUROPE
Enzo Life Sciences (ELS) AG
Industriestrasse 17
CH-4415 Lausen
Switzerland
Phone:+41/0 61 926 89 89
Fax:+41/0 61 926 89 79
info-ch@enzolifesciences.com

For local distributors and detailed product information visit us online:
www.enzolifesciences.com