AMPIVIEW™ SARS-CoV-2 RNA probes with SAVIEW® AP/Red Kit

REF ENZ-GEN158 20 tests

INTENDED USE:

For Research Use Only. Not for use in diagnostic procedures.

SUMMARY AND EXPLANATION

AMPIVIEW™ RNA probes are uniquely designed with the precision of targeted, sequence-specific RNA probes, powered by Enzo's LoopRNA ISH™ technology to deliver superior sensitivity and compatible with existing nanopolymer detection systems used in immunohistochemistry (IHC) procedures.

AMPIVIEW™ SARS-CoV-2 RNA probes is a mix of RNA probes that targets the nucleocapsid (N) protein and spike (S) protein of SARS-CoV-2. Coronavirus disease (COVID-19) is caused by SARS-CoV-2 virus and it's known to affect the lower respiratory system.

ASSAY PRINCIPLE

The biotin-labeled probe mixture will denature and hybridize to the fixed and pre-treated tissue section on the microscope slide. The labeled probes can be detected with a streptavidin detection system such as Enzo's SAVIEW® PLUS reagents (AP or HRP) combined with HIGHDEF® chromogens. Moreover, an unconjugated biotin antibody can be combined with Enzo's nanopolymer detection system such as Enzo's POLYVIEW® PLUS reagents. Results can be visualized under a light microscope. For more information and other detection solutions available, visit *enzolifesciences.com/IHC*.

KNOWN APPLICATION

in situ Hybridization (ISH) on formalin-fixed paraffin-embedded (FFPE) tissue specimens or cells.

PRODUCTS SUPPLIED

AMPIVIEW™ SARS-CoV-2 RNA probes (ENZ-GEN157)
AMPIVIEW™ Hybridization Buffer (ENZ-ACC152)
AMPIVIEW™ Wash Buffer 1
AMPIVIEW™ Wash Buffer 2
SAVIEW® PLUS AP (Store at 4°C)
HIGHDEF® Red AP Chromogen/Substrate
Proteinase K (ENZ-33801)
HIGHDEF® Hematoxylin (ENZ-ACC106)

MATERIALS NEEDED (Not Provided)

Materials not provided are PAP pen (ADI-950-233), xylene or xylene substitute, 100% reagent grade ethanol, distilled or deionized water, Tris Buffered Saline with Tween (TBS-T) (20 mM Tris pH 7.6, 150 mM NaCl, 0.05% Tween-20), heating blocks for slides and heating oven.

STORAGE AND SHELF-LIFE

- Upon receipt, store probes solution and hybridization buffer at -20°C. For long-term storage, you can store probes at -80°C. These products are stable under these conditions up to the expiration date indicated in the vial label.

PERFORMANCE CONSIDERATIONS

1. Do not use reagents past their expiration date.
2. Do not allow the slides to dry completely during the hybridization and detection procedures, or erroneous results may occur. Avoid drying by ensuring that the entire specimen is covered with sufficient amounts of buffers and reagents as recommended in the procedures. While incubating, the slides may be covered with a coverslip to help prevent drying.
3. Cross-contamination of samples could cause false results. Use care preparing slides for more than one specimen.
4. Allow all components to reach room temperature (20-30°C) before beginning the test procedure.
5. Incubation times and temperatures other than those specified may give erroneous results.
6. Improper specimen preparation may cause false results.

CONTROLS

To assure the staining procedures are performed correctly, a control slide should be run with the first set of specimen slides. It serves as a hybridization/detection control and as an aid in interpretation of the specimen slides. Each laboratory can prepare tissue control slides from known HPV positive tissue blocks. If the control slides do not appear as expected, the test run should be invalid.

LIMITATIONS

- This procedure is for research use only. It is not intended for diagnostic or therapeutic use.
- Negative results do not rule out the possibility of SARS-CoV-2 infection.
- It has been reported that there is a marked variability in the number of cells containing SARS-Cov-2.The majority of cell types involved are macrophages.

PRECAUTIONS

1. Refer to reagent Safety Data Sheet (SDS) from precautions.
2. Specimens, before and after fixation, and all materials exposed to them should be handled and disposed of with proper precautions.

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3. Never pipette reagents by mouth and avoid contact with skin and mucous membranes with reagents and specimens. If reagents and/or specimens come into contact with sensitive areas, rinse thoroughly with water and follow your institution's safety protocols.

TECHNICAL NOTES

1. AMPIVIEW™ RNA probes have been optimized with SAVIEW® PLUS detection solutions.
2. AMPIVIEW™ RNA probes have been optimized with anti-biotin (mouse or rabbit) and POLYVIEW® PLUS Detection Systems. Use of other polymer-based reagents will require prior optimization.
3. Autostainers such as LEICA Bond III or Bond Max, Ventana Discovery Ultra or others can be used, but will require prior optimization.
4. Changes in the amount of probe or temperature incubation times from what's recommended may lead to inconsistent results.

INSTRUCTIONS FOR USE

AMPIVIEW™ SARS-CoV-2 RNA probes are developed for manual and automated systems in combination with a streptavidin-based IHC detection system such as Enzo's SAVIEW® PLUS detection reagents and HIGHDEF® chromogens.

The following protocol offer the optimized conditions for manual performance.

1. Bake slides in a dry oven for 35-40 minutes at 65°C.

Deparaffinization

2. Formalin-fixed paraffin-embedded (FFPE) tissue sections must be deparaffinized with xylene, xylene substitute or dewaxing agent before rehydrating the sample with a series of ethanol and water washes.

Step	Solution	Time
1	Xylene [substitute]	10 minutes
2	Xylene [substitute]	2 minutes
3	100% Ethanol	1 minute
4	100% Ethanol	1 minute
5	90% Ethanol	1 minute
6	70% Ethanol	1 minute
7	50% Ethanol	1 minute
8	Deionized water	1 minute

3. Use a pap pen to mark around the tissue section.

   Prepare the proteinase K (ENZ-33801) for the next step. For tissue we use 40 µg/mL proteinase K diluted in DPBS.

4. Transfer slides into 1X TBS-T wash buffer for 5 minutes at room temperature.
5. To each specimen add a generous amount (from 300 µL to 500 µL) of freshly prepared Proteinase K (80µg/mL) and incubate 37ºC for 10 minutes.
6. Transfer the slides sequentially in a new glass or plastic jar containing 1X TBS-T wash buffer for 2 to 5 minutes and then in distilled water for 1 minute.
7. Dehydrate the slides sequentially.

Step	Solution	Time
1	50% Ethanol	1 minute
2	70% Ethanol	1 minute
3	100% Ethanol	1 minute

8. Place the slides on heating block at 37°C to dry 100% alcohol drops.

Hybridization

9. Add 50 to 100 µL AMPIVIEW™ SARS-CoV-2 RNA probes onto dried slide and cover the specimen with a cover slip.
10. Denature samples at 60°C for 5 minutes.
11. Place the slides in the hybridization oven at 40°C for 2 hours.

Post-hybridization and Detection

12. After the 2 hours, transfer the slides to a jar containing 1X TBS-T wash Buffer and leave it till the coverslips slide off naturally (3-5 minutes).
13. Tap off excess of wash buffer and place the slides on a heating block at 42ºC and immediately add 300 µL of pre-heated (42ºC) AMPIVIEW™ Wash Buffer 1 (ENZ-ACC153) for 7 minutes.
14. Tap off excess and place slides in heating block at 42°C and immediately add 300 µL of pre-heated (42ºC) AMPIVIEW™ Wash Buffer 2 (ENZ-ACC154) for 7 minutes.
15. Transfer the slides into a jar containing 1X TBS-T wash buffer for 3-5 minutes.
16. Removing one slide at a time, tap off excess wash buffer and add approximately 3-4 drops of SAVIEW® PLUS AP reagent and incubate for 30-40 minutes at room temperature.
17. Wash in 1X TBS-T wash buffer for 5 minutes.

    During the last wash step, prepare chromogen by mixing 150 µL substrate with 8 µL of red chromogen per slide.

18. Tap off excess of wash buffer. Apply 158 µL of HIGHDEF® Red AP chromogen solution and incubate for 15 minutes at room temperature.
19. Wash with 1X TBS-T wash buffer for 5 minutes.
20. Wash with distilled water for 3-5 minutes.
21. Counterstain with Hematoxylin II for 3-5 minutes.
22. Wash with tap water.
23. Transfer slides in distilled water.
24. Let slides air dry before applying any mounting medium.

Perform post-hybridization processing and detection according to detection kit.

For specific protocols, visit enzolifesciences.com/AMPIVIEW

INTERPRETATION OF RESULTS

Using SAVIEW® PLUS detection systems and HIGHDEF® chromogens, hybridized AMPIVIEW™ SARS-CoV-2 RNA probes

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appear as [chromogen color] pattern when detected with SAVIEW® PLUS AP or HRP.

Please note:

- Do not evaluate areas of necrosis, overlapping nuclei, over-digested nuclei, nuclei with weak signal intensity.
- A negative or unspecific result can be caused by multiple factors.
- In order to correctly interpret the results, the used must validate this product prior to use.

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