AMPIVIEW™ NORAD (AS) Dig RNA probes (Mouse) Set

REF ENZ-GEN178-2000 2 x 1.0 mL AMPIVIEW™ NORAD (AS) Dig RNA Probes (2 µg/mL) (Mouse)
1 x 2.0 mL AMPIVIEW™ Hybridization Buffer (1X)

INTENDED USE:

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

SUMMARY AND EXPLANATION

Powered by Enzo's LoopRNA ISH™ technology to deliver superior sensitivity, **AMPIVIEW™ RNA probes** are uniquely designed to specifically hybridize to RNA, DNA or RNA/DNA targets on tissue sections and cells, and are compatible with Enzo's complete immunohistochemistry (IHC) detection solutions, from antigen retrieval to counterstain.

AMPIVIEW™ NORAD (AS) Dig RNA probes are specifically designed to detect mouse or rat non-coding RNA activated by DNA damage (Norad) long non-coding RNA (lncRNA). Norad is a broadly expressed, highly abundant, and conserved mammalian lncRNA that is induced after DNA damage. LncRNAs are identified as critical regulators in cerebral ischemia/reperfusion injury [1] and are known to play an important role in the process of aging mammals [2]. The probe hybridizes to tissue sections fixed and pre-treated on microscope slides.

ASSAY PRINCIPLE

AMPIVIEW™ NORAD (AS) Dig RNA probes are digoxigenin-labeled antisense probes that will hybridize to the fixed and pre-treated tissue section on the microscope slide. The probes can be detected with an unconjugated anti-digoxigenin linker, followed by a polymer-based detection solution such as Enzo's POLYVIEW® PLUS reagents combined with HIGHDEF® chromogens and counterstain. Results can be visualized under a light microscope. For more detection product information visit enzolifesciences.com/AMPIVIEW.

KNOWN APPLICATION

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

PRODUCTS SUPPLIED

AMPIVIEW™ NORAD (AS) Dig RNA probes (2µg/ml) (ENZ-GEN177)
AMPIVIEW™ Hybridization Buffer (1X) (ENZ-ACC152)

MATERIALS RECOMMENDED FOR PRE-TREATMENT AND DETECTION (Not Provided)

Antigen Retrieval Reagent, pH 9.0 (10X) (ENZ-ACC113)
Proteinase K (ENZ-33801)
POLYVIEW® PLUS (anti-rabbit) Detection Reagents (AP or HRP)
HIGHDEF® Chromogen/Substrate
HIGHDEF® Hematoxylin (ENZ-ACC106)
DIGX® rabbit anti-digoxigenin linker (ENZ-ABS303)
AMPIVIEW™ Wash Buffers Kit (ENZ-ACC161)
AMPIVIEW™ Ubiquitin and NSP Dig Controls Kit (ENZ-KIT223)

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

Visit enzolifesciences.com/IHC for more specific POLYVIEW® PLUS detection solutions and Enzo's unrivaled selection of high-definition chromogens.

STORAGE AND SHELF-LIFE

Upon receipt, store probes solution and hybridization buffer at -20°C. For long-term storage, 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 cover slip 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. AMPIVIEW™ Ubiquitin (AS) DIG RNA probes can be used as positive control probes for high to medium expressing targets. AMPIVIEW™ NSP-DIG RNA probes can be used as negative control probes If staining on 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.

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TRADEMARKS AND PATENTS

Several Enzo Life Sciences products and product applications are covered by US and foreign patents and patents pending. Enzo is a trademark of Enzo Life Sciences, Inc.

PRECAUTIONS

1. Refer to reagent Safety Data Sheet (SDS) for precautions.
2. Specimens, before and after fixation, and all materials exposed to them should be handled and disposed of with proper precautions.
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.
4. Avoid RNases, which are commonly found on skin. Wear gloves at all times, and work in a clean environment.

TECHNICAL NOTES

1. **AMPIVIEW™ NORAD (AS) Dig RNA probes** set come with as a ready-for-use, 2 µg/ml concentration probes against human NORAD and hybridization buffer to adjust concentration depending on protocol used.
2. **AMPIVIEW™ RNA probes** have been optimized with DIGX® anti-digoxigenin (mouse or rabbit) and POLYVIEW® PLUS Detection Systems in combination with HIGHDEF® chromogens. Use of other linkers and polymer-based reagents will require prior optimization.
3. Autostainers such as LEICA BOND, Roche DISCOVERY or other automated systems can be used, but will require prior optimization. AMPIVIEW™ NORAD (AS) Dig RNA probes might need to be diluted with the hybridization buffer for automated systems.
4. Changes in the amount of probe or temperature incubation times from what is recommended without prior testing, may lead to inconsistent results.

INSTRUCTIONS FOR USE

The following protocol is for manual processing of FFPE specimens with **AMPIVIEW™ NORAD (AS) DIG RNA probes** in combination with Enzo's DIGX® anti-digoxigenin linker, POLYVIEW® PLUS detection reagents, HIGHDEF® chromogens and counterstain.

Set water bath at 85ºC ± 3°C, heating block at 37°C and hybridization oven at recommended temperature for your probes.

1. Bake slides in a dry oven for 60 minutes at 60°C.

Deparaffinization and Antigen Retrieval

Ensure all containers remain covered.

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	100% Ethanol	2 minutes

NOTE: Use fresh reagents in each jar for each batch of slides and discard in appropriate waste container when finished.

Antigen Retrieval

Prepare 1X antigen retrieval solution by mixing 10 mL of Antigen Retrieval Reagent, pH 9.0 (10X) (ENZ-ACC113) with 90 mL of deionized water to make 100 mL of solution.

3. Put slides in a jar filled with sufficient pre-heated (82ºC) retrieval solution to cover the tissue sections on the slides.
4. Place the jar in a pre-heated (85ºC ± 3°C) water bath and incubate slides with the antigen retrieval solution for 30 minutes*. Remove the jar and cool down slides using distilled water for 1 minute.
   *Optimal antigen retrieval solution incubation time and temperature should be determined by the end user depending on the nature of the tissue specimen.

5. Dry slides in 100% ethanol for 3 minutes.
6. Use pap-pen to mark around the sections.

NOTE: after this step, tissue can be stored overnight at 4°C for FFPE or -80°C for frozen sections.

For tissue specimens, prepare the proteinase K (ENZ-33801) for the next step. For tissue, 40 µg/mL proteinase K diluted in TBS-T is recommended.

► When using HRP detection reagent, treat tissue samples with fresh 3% peroxide (H_2O_2) for 10 minutes at room temperature.

7. To each specimen add a generous amount (from 300 µL to 500 µL) of freshly prepared Proteinase K (40 µg/m)** and incubate at 42ºC for 10 minutes.

   **Optimal proteinase K concentration and time should be determined by the end user depending on the nature of the tissue specimen.

8. Wash the slides in a new glass/plastic jar containing RNase free water for 5 minutes.
9. Pre-condition the tissue with hybridization buffer for 2 minutes.

Hybridization

10. Remove hybridization buffer from sample and add 30 to 100 µL **AMPIVIEW™ NORAD (AS) DIG RNA probes** onto slide and cover the specimen with a cover slip.
    (Optional, for DNA/RNA detection) Denature samples at 80°C for 5 minutes.
11. Place the slides in the hybridization oven at 42°C ± 2°C for 2 hours.

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Post-hybridization and Detection with POLYVIEW® PLUS solutions combined with HIGHDEF® chromogen and counterstain.

12. After the 2 hours, transfer the slides to a jar containing 1X TBS-T and leave it till the coverslips slide off naturally (3-5 minutes).
13. Tap off excess of TBS-T 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. Add 3-4 drops of DIGX® rabbit anti-digoxigenin linker (RTU) and incubate for 20 to 60 minutes at room temperature.
17. Wash with 1X TBS-T wash buffer for 5 minutes.
18. Apply 3-4 drops of the POLYVIEW® PLUS AP (anti-rabbit) or HRP reagent for 30 minutes at room temperature.
19. Wash in 1X TBS-T wash buffer for 5 minutes.
20. During the last wash step, prepare chromogen according to product protocol.
21. Tap off excess of wash buffer. Apply 150 µL of HIGHDEF® AP or HRP chromogen solution (depending on the detection reagent) and incubate for 15 minutes at room temperature.
22. Wash with 1X TBS-T wash buffer for 5 minutes.
23. Wash with distilled water for 3-5 minutes.
24. Counterstain with 50% Hematoxylin diluted with dH_2O for 3-5 minutes.

    NOTE: Diluted product should not be stored more than a week.

25. Wash with tap water.
26. Mount sections with coverslip with no air bubbles Samples are ready to be visualized under the light microscope.

For specific protocols, visit enzolifesciences.com/AMPIVIEW

INTERPRETATION OF RESULTS

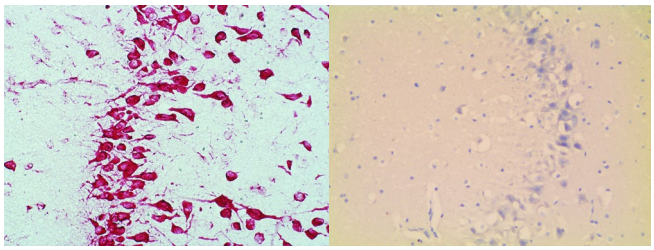
Using POLYVIEW® PLUS detection systems and HIGHDEF® chromogens, hybridized AMPIVIEW™ NORAD (AS) DIG RNA probes signal appears as red in the tissue specimen (Figure 1A), while tissue specimens with negative control probes or no probes do not show any red signal (Figure 1B).

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.

Figure 1

A B



NORAD detection in mouse brain tissue specimen with A. AMPIVIEW™ NORAD (AS) Dig RNA probes and B. AMPIVIEW™ NSP-Dig RNA probes (negative control), detected with DIGX® rabbit anti-digoxigenin linker (ENZ-ABS303), POLYVIEW® PLUS AP (anti-rabbit) (ENZ-ACC110) combined with HIGHDEF® Red AP Substrate/Chromogen and hematoxylin.

For more information, visit enzolifesciences.com/AMPIVIEW

REFERENCES

1. Liu D, *et al.* Upregulated LncRNA NORAD can diagnose acute cerebral ischemic stroke patients and predict poor prognosis. *Folia Neuropathol,* 2023.
2. Barry G, *et al.* Long Non-Coding RNA Expression during Aging in the Human Subependymal Zone. *Frontiers in Neurology* 2015.

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