HPV Type 31 Probe (Digoxigenin)

Hybridization Probe
902-4051-030321

BIOCARE MEDICAL

Available Product Formats			
Format	Catalog Number	Dilution	Diluent
Manual	BRR 4051 A, -0.6	1:5 to 1:10	DNA Hybridization Buffer
ONCORE Pro	BPR 4051 T60	1:5	DNA Hybridization Buffer

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

Summary and Explanation:
HPV Type 31 Probe is a digoxigenin-labeled DNA probe at a size range between 60 and 110 base pairs. This probe will hybridize to HPV Type 31 sequences in formalin-fixed paraffin-embedded tissues (FFPE).

HPV is a double stranded DNA virus containing approximately 7,900 base pairs in its genome. There are approximately 120 different types of known HPVs; however, relatively few are capable of causing cancerous lesions in humans. Viral types can be separated into low or high risk. Low risk types (e.g. 1/2/3/4/6/7/10/11/42/44/63) typically cause warts (verracue) while high risk types (e.g. 16/18/31/33/35/39/45/51/52/56/58/59/82) have been closely linked to head / neck cancers, cervical intraepithelial (CIN) lesions and cervical malignancies. HPVs primarily infect the basal cells of the stratified epithelium. Virions gain access through trauma or micro abrasions in the skin.

Principle of Procedure:
This digoxigenin-labeled probe will hybridize to its specific DNA target in cells infected with HPV. The labeled probe is detected with an unconjugated mouse anti-digoxigenin antibody, followed by a goat anti-mouse HRP polymer conjugate and visualization with a colorimetric reaction.

Known Applications:
in situ hybridization (formalin-fixed paraffin-embedded tissues (FFPE))

Supplied As:
HPV Type 31 Probe is a digoxigenin-labeled DNA probe designed to hybridize to HPV Type 31 DNA sequences.

HPV Type 31 Probe (0.5 ng/µL) is provided in DNA Hybridization Buffer (BRI4036) containing dextran sulfate, nucleic acid carriers and formamide.

Materials and Reagents Needed But Not Provided:
Reagents and materials, such as detection kits and ancillary reagents are not provided.

Refer to the Biocare Medical website located at http://biocare.net for information regarding catalog numbers and ordering.

Refer to the ONCORE Pro Automated Slide Staining System User Manual for a complete list of ONCORE Pro specific materials and reagents required.

Species Reactivity: HPV Type 31 DNA

Cellular Localization: Nuclear

Storage and Stability:
Store at 2°C to 8°C. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

Instructions for Use (Manual use):

DAY 1

1. Deparaffinization
a) Deparaffinize slides in Xylene as per standard procedures.
b) Perform 5 minute hydrogen peroxide block.
c) Wash with distilled water, and place onto IQ Stainer at room temperature (RT).

2. Protein Digestion/ Retrieval
The following recommendations should be used as a starting point for tissues fixed 24 hours or longer. Tissues fixed less than 24 hours may require a further dilution of Pronase concentrate to buffer and/ or a heat pretreatment at lower temperature.
a) Prepare Pronase working solution by combining 1 part enzyme to 9 parts buffer. (Working solution is stable for 14 days at 4°C.)
b) Place 4 drops onto tissue sample for 10 minutes at RT.
c) Wash in distilled water.
d) Preheat Diva Decloaker retrieval solution to 95°C in Biocare's Decloaking Chamber™. Retrieve section with Diva Decloaker using Biocare's Decloaking Chamber™, followed by a wash in distilled water.
   i. Suggested parameters: 95°C for 25 minutes, followed by 5 minute cool down in the retrieval solution.
   ii. Wash in distilled water.
   iii. Air dry slides lightly.

3. Probe Hybridization
a) Ensure probe is thoroughly mixed by inverting several times or briefly vortexing.
b) Apply 20 µl of digoxigenin-labeled probe onto tissue section and cover slip with 22×22mm cover slip.
c) Denaturation step: Place slides onto an IQ Stainer or a hot plate at 95°C for 5 minutes.
d) Hybridization step: Place slides onto an IQ Stainer or humidity chamber at 40°C for 16-24 hours.

DAY 2

4. Post-Hybridization Washing
a) Pour sufficient amount of SSC Wash Buffer directly onto the slides on 40°C IQ Stainer. Wait 5-10 minutes until cover slips loosen up. Carefully move cover slips sideways and remove.
b) Flood the slides with SSC Wash Buffer and incubate for 5 minutes on 40°C IQ Stainer. Do not agitate. Do not let tissues dry out during post-hyb washing. (Post-hyb washing may also be done in 40°C water bath).
c) Wash slides in RT TBS Wash Buffer.

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5. Detection of Probe

a) Decant TBS and add 4 drops of Mouse anti-Digoxigenin onto tissue sample. Incubate for 15 minutes.
b) Wash with TBS buffer.
c) Decant TBS and add 4 drops of MACH 2 Mouse HRP-Polymer onto tissue sample. Incubate for 15 minutes.
d) Wash with TBS buffer.
e) Decant TBS, apply 4 drops of Deep Space Black Chromogen working solution (prepared according to chromogen kit data sheet) onto tissue sample. Incubate for 5 minutes.
f) Wash with distilled water.

6. Counterstaining

a) Briefly soak slides in CAT Hematoxylin for 5-6 seconds. Immediately rinse with distilled water.
b) Soak slides in Tacha's Bluing Solution for 5-6 seconds, and rinse with distilled water.

7. Cover Slipping

a) Dehydrate through graded alcohols and finish in xylene.
b) Apply 1 drop of mounting media to an appropriate cover slip and mount.
c) Allow to dry.

Instructions for Use (ONCORE™ Pro Automated Slide Staining System):

BPR4051 is intended for use with the ONCORE Pro. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:
Protocol Name: create in Reagent Editor*
Protocol Template (Description): CISH DNA 1 Template 1
Reagent Name, Time, Temp.: ISHzyme**, 15 min., 25°C

Dilute BPR4051 into 15 mL ONCORE Pro Improv Vial (ONCPR102). Refer to the ONCORE Pro Automated Slide Staining System User Manual (RFID Editor) for instructions on proper use.

*When creating probe name in Reagent Editor, select Viscosity 2 and check as Hazardous.

**Recommended mixing ratio for ISHzyme (OPRI6039K) is 1:50. Incubation time may be reduced if tissue morphology is impaired.

Baking Slides Before Staining for 10 min at 60°C on ONCORE Pro is recommended to improve tissue retention.

Technical Notes:

1. The Biocare ISH HRP Detection Kit for Digoxigenin-Labeled DNA Probes (BRI4050K) has been developed to detect Biocare's digoxigenin-labeled probes by in situ hybridization. ONCORE Pro ISH HRP Detection Kit (OPRI6047K) is designed to be used on ONCORE Pro Automated Slide Staining System with digoxigenin-labeled probes. Routinely processed (FFPE) tissues in which the presence of cognate nucleic acid target is anticipated can be used.
2. Biocare's IQ Kinetic Slide Stainer may be used for hybridization and post-hybridization detection steps of digoxigenin-labeled probes. Detection steps can also be performed on Biocare's intelliPATH™ Automated Slide Stainer with intelliPATH™ DAB Chromogen Kit (IPK5010).
3. If using commercially available humidity chambers, hybridize probe for 16-24 hours. Both incubator and humidity chamber must be at 40ºC when hybridizing probe. Other hybridization chambers can be used, but

Technical Notes Cont'd:

measures should be taken to ensure that chamber is hermetically sealed during hybridization.
4. To reduce non-specific background, Background Punisher (BP974) may be used for 10 min after Mouse anti-Digoxigenin.
5. The IQ Stainer can be used as an incubation and humidity chamber by using the IQ Aqua Sponge. Saturate IQ Aqua Sponge with distilled water, and place on hot bar set to 40°C for hybridization. Use the clear plastic hood to contain heat and moisture. Commercially available solutions for fog prevention may be used to prevent excessive accumulation of the moisture inside the clear plastic hood.
6. The use of probe in amounts less than recommended may lead to inconsistent results.
7. HPV Type 31 may cross react with uncharacterized novel viral types. It is the responsibility of the user to validate their test.

Limitations:

This product is provided for Research Use Only (RUO) and is not for use in diagnostic procedures. Suitability for specific applications may vary and it is the responsibility of the end user to determine the appropriate application for its use.

Precautions:

1. Refer to reagent Safety Data Sheet for precautions.
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. (6)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.

HEALTH HAZARD

Technical Support:

Contact Biocare's Technical Support at 1-800-542-2002 for questions regarding this product.

References:

1. Nuovo GJ. In Situ Molecular Pathology and Co-Expression Analyses. 1st Ed. San Diego: Academic Press, 2013.
2. Wilkinson DG. In Situ Hybridization: A Practical Approach (Practical Approach Series). 2nd Ed. Oxford: Oxford University Press, 1999.
3. Nuovo GJ. A comparison of subgenomic and genomic DNA probes for detection and typing of human papillomavirus by in situ hybridization. J Histotech. 1995; 18:105-110.
4. Nuovo GJ. Determination of HPV type by in situ hybridization analysis: A comparative study with Southern blot hybridization and the polymerase chain reaction. J Histotech. 1992; 15:99-104.
5. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
6. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

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