

RNAscope[™] ISH Probe High Risk HPV

REF 200450

For *in vitro* diagnostic use.
For US export only.

INTENDED USE

RNAscope ISH Probe High Risk HPV is used in an *in situ* hybridization (ISH) assay for the qualitative detection of HPV E6/E7 mRNA in formalin-fixed paraffin-embedded (FFPE) tissue specimens by light microscopy. The assay detects high-risk HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82. RNAscope ISH Probe High Risk HPV is for use in clinical laboratories with BOND RNAscope Brown Detection on the automated Leica Biosystems BOND-III stainer.

RNAscope ISH Probe High Risk HPV is indicated for use for patients diagnosed with oropharyngeal squamous cell carcinoma (OPSCC) to aid in the identification of high-risk HPV. The clinical interpretation of any hybridization signal or its absence should be performed by a qualified pathologist with proper controls, complemented by histological examination and relevant clinical information.

PRINCIPLE OF THE PROCEDURE

RNAscope ISH Probe High Risk HPV contains oligonucleotide probes designed to hybridize to high-risk HPV E6/E7 nucleic acid sequences in FFPE tissue sections.¹ The probe is visualized using the detection reagents of BOND RNAscope Brown Detection, resulting in a brown chromogenic signal that can be evaluated using a light microscope.^{2,3} Positive and negative control probes are used to assess RNA integrity in the sample and evaluate background and/or non-specific staining, respectively.

REAGENT PROVIDED

RNAscope ISH Probe High Risk HPV is provided as a 14 mL ready-to-use solution of oligonucleotide probe in hybridization buffer containing formamide. The quantity provided is sufficient to perform 30 tests. No reconstitution, mixing, dilution or titration is required.

STORAGE AND STABILITY

- Store probe at 2-8 °C upon receipt and immediately after use.
- Unopened probe is stable until the expiration date printed on the bottle. Do not use after the expiration date.
- The probe is stable for at least 3 weeks after transfer to a BOND Open Container when stored at 2-8 °C after use.

MATERIALS REQUIRED BUT NOT PROVIDED

- RNAscope ISH Probe UBC (Positive Control) (Cat. No. 200460)
- RNAscope ISH Probe dapB (Negative Control) (Cat. No. 200470)
- BOND RNAscope Brown Detection (Leica Biosystems, DS9815)
- BOND Open Container 30 mL (Leica Biosystems, OP309700)
- BOND Epitope Retrieval 2 (Leica Biosystems, AR9640)
- BOND Dewax Solution (Leica Biosystems, AR9222)
- 10X BOND Wash (Leica Biosystems, AR9590)
- BOND-III Slide Stainer (Leica Biosystems) running BOND software v5.1 or above with BDD v82 or above.



The “Using BOND Reagents” section in the BOND User Manual identifies common materials required to perform the procedure on the BOND instrument.

TEST SPECIMENS

Specimens must be formalin-fixed, paraffin-embedded (FFPE) human oropharyngeal carcinoma tissue. Fix tissues in 10% neutral-buffered formalin (NBF), and section tissue blocks at 4-5 µm thickness. Stain the specimens within 3 weeks of mounting on slides when stored at room temperature (20-25 °C).

STAINING PROCEDURE

Specimen staining should be performed by laboratory personnel trained in histological procedures and use of the BOND-III instrument.

Positive and Negative Tissue Controls

Stain separate HPV-positive and HPV-negative FFPE tissue controls with the RNAscope ISH Probe High Risk HPV for each automated stainer run of patient specimens. Control tissues are supplied by the user based on prior testing with RNAscope ISH Probe High Risk HPV. These controls verify that the test produces the expected positive and negative results with known HPV-positive and HPV-negative samples tested with the HPV target probe.

Positive and Negative Control Probes with each Patient Sample

Test every patient specimen with positive and negative control probes on adjacent serial sections from the same tissue block used for the RNAscope ISH Probe High Risk HPV. The test requires 3 slides from every patient specimen: one for the positive control probe, one for the negative control probe, and one for the HPV target probe.

Positive Control Probe

The positive control probe evaluates the integrity of RNA in the sample by measuring the presence of RNA from a common housekeeping gene. The recommended positive control probe is RNAscope ISH Probe UBC (Positive Control) (Cat No. 200460), which targets the mRNA transcript of the human ubiquitin C (UBC) gene (342-1503, Accession # NM_021009).

Negative Control Probe

The negative control probe evaluates the specificity of any observed staining with the High Risk HPV probe and is used to assess background or nonspecific staining in the sample. The recommended negative control probe is RNAscope ISH Probe dapB (Negative Control) (Cat No. 200470), which targets the mRNA transcript of the dihydrodipicolinate reductase (dapB) gene (414-862; Accession # EF_191515) of *Bacillus subtilis*.

Performing the Procedure

1. Transfer the RNAscope ISH probe to a new, unused BOND Open Container (30 mL).
2. Follow the instructions for the BOND instrument to register reagents and probes, create cases and slides, label slides, load reagents, probes, and slides, and start the run.



3. Use the following protocols for staining HPV and control probes:

Protocol Type	Protocol Name
Staining	*RNAscope DAB ISH Protocol B
Preparation	*Bake and Dewax
HIER	*RNAscope Target Retrieval (95)
Enzyme	*RNAscope Enzyme
Denaturation	---
Hybridization	*RNAscope Hybridization

Note: The “*” in each protocol name is included in the BOND software to denote protocols that are provided from Leica Biosystems.

4. At the completion of the run, unload slides from the BOND stainer, dehydrate in alcohol and xylene, and coverslip according to the established laboratory process.

INTERPRETATION OF RESULTS

Interpretation of specimens should be performed by a qualified anatomic pathologist.

Tissue Run Controls

Run validity is determined using HPV-positive and HPV-negative tissue controls following the algorithm in Figure 1.

1. Examine the HPV-positive tissue control slide under a 20X or 40X objective lens. An acceptable result is unequivocal staining (large nuclear brown dots and/or granular nuclear/cytoplasmic brown dots) in tumor cells, with staining present diffusely across the tumor in the context of adjacent negative staining within non-tumor squamous epithelium and in non-tumor stromal cells, fibroblasts, lymphocytes, and endothelial cells, while discounting artifactual staining.
2. Examine the HPV-negative tissue control slide under a 20X or 40X objective lens. An acceptable result is the absence of unequivocal staining within tumor cells.
3. The run is valid if both the HPV-positive and HPV-negative tissue control slides are acceptable.
4. The run is invalid if either the HPV-positive or HPV-negative tissue control slides are not acceptable.

Examination and Interpretation of Patient Specimen Results:

Interpret results following the scoring algorithm in Figure 2 to determine if the sample is positive or negative for HPV. Assessment of the HPV probe slide results should be followed by interpretation of the positive and negative probe controls (UBC and dapB probes) as described.

1. Using a hematoxylin & eosin (H&E) stain, confirm that the specimen is squamous cell carcinoma with a minimum of 50 tumor cells present to be suitable for testing with RNAscope ISH Probe High Risk HPV.
2. Examine the target probe (RNAscope ISH Probe High Risk HPV) slide under a 20X objective lens. If staining is not readily seen under the 20X objective, examine the slide under a 40X objective lens.



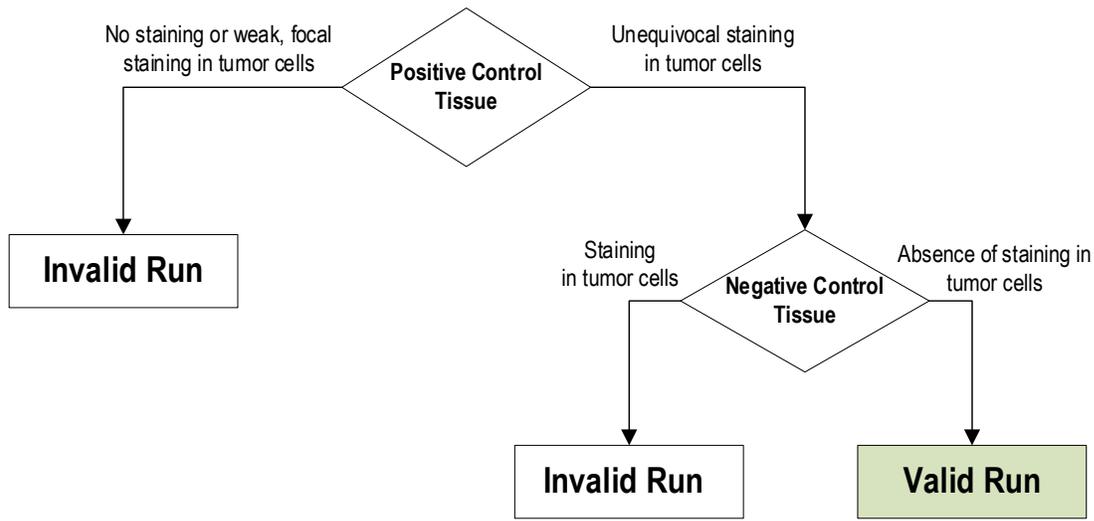


Figure 1: Interpretation of positive and negative tissue controls stained with RNAscope ISH Probe High Risk HPV.

3. A positive High Risk HPV ISH result requires the finding of unequivocal staining (large nuclear brown dots and/or granular nuclear/cytoplasmic brown dots) in tumor cells, with staining present diffusely across the tumor in the context of adjacent negative staining within non-tumor squamous epithelium and in non-tumor stromal cells, fibroblasts, lymphocytes, and endothelial cells, while discounting artifactual staining. Substantial artifactual staining of tumor and/or non-tumor cells of epithelial or non-epithelial origin may preclude interpreting a positive result.

If unequivocal staining is present in the High Risk HPV slide, evaluate the dapB slide as follows:

- i. If there is no staining or only weak and/or focal staining in tumor cells, the sample is reported as HPV positive.
- ii. If staining is present in tumor cells with signal present diffusely across the tumor, the sample is reported as indeterminate for HPV.

4. A negative High Risk HPV ISH result is the absence of unequivocal staining (neither large nuclear brown dots nor granular nuclear/cytoplasmic brown dots) within tumor cells, in the context of acceptable sample quality as determined by the UBC positive control.

If unequivocal staining is not present in the High Risk HPV slide, evaluate the UBC slide as follows:

- i. If staining is present in tumor cells with signal present diffusely across the tumor, the sample is reported as HPV negative.
- ii. If there is no staining or only weak, focal staining in tumor cells, the sample is reported as indeterminate for HPV.



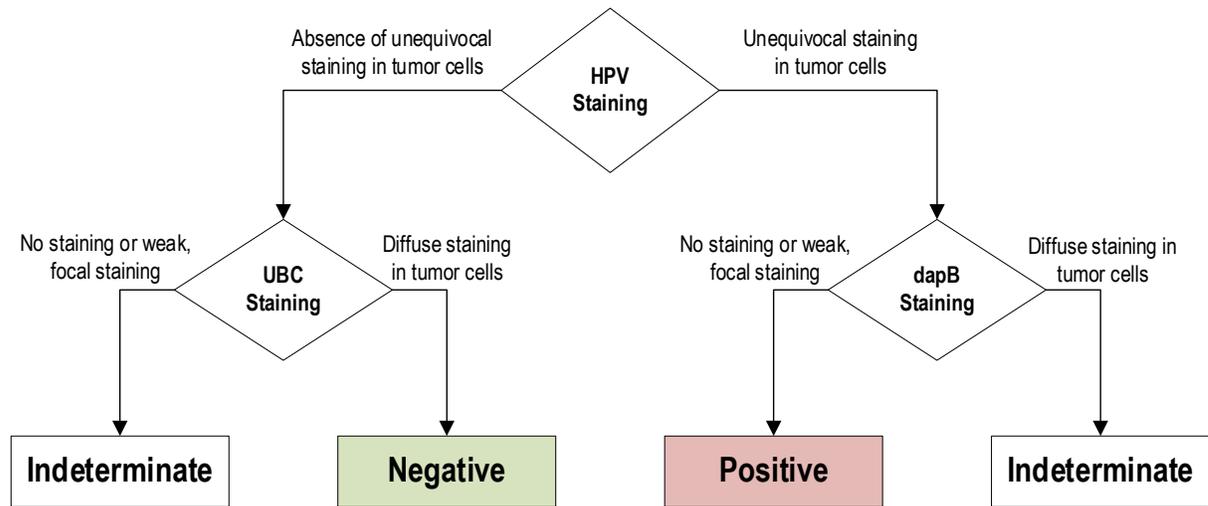


Figure 2: Interpretation of the RNAscope HPV test

PERFORMANCE CHARACTERISTICS

Analytical Specificity

Analytical specificity was tested to measure the ability of the assay to detect the high-risk HPV subtypes (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82), and not detect HPV low-risk subtypes (6, 11, 40, 42, 43 and 44). Plasmids containing the E6/E7 region of the HPV genome for each HPV subtype were spotted and cross-linked to glass slides. RNAscope ISH Probe High Risk HPV was tested on the plasmid slide. All 18 high-risk subtypes were detected by RNAscope ISH Probe High Risk HPV. No cross-reactivity was observed with low-risk HPV subtypes.

Analytical Precision

The reproducibility of staining performance of RNAscope ISH Probe High Risk HPV was assessed across multiple days, different instruments, and different probe lots.

Inter-day precision was conducted over five days, using one probe lot and one BOND-III instrument. Three tissue sections of each of five cases of oropharyngeal carcinoma (3 positive, 2 negative) were stained on each day. The overall percent agreement for ISH signal between days was calculated as 100% for 75 sections evaluated. Intra-day repeatability was also assessed. The overall percent agreement for ISH signal within runs was calculated as 100% for 75 sections evaluated.

Inter-instrument precision was conducted using one lot of probe and three BOND-III instruments. Three tissue sections of each of five cases of oropharyngeal carcinoma (3 positive, 2 negative) were stained on each instrument. The overall percent agreement for ISH signal between instruments was calculated as 100% for 45 sections evaluated.

Inter-lot precision was conducted using three probe lots. Two tissue sections of each of five cases of oropharyngeal carcinoma (3 positive, 2 negative) were stained on each instrument. The overall percent agreement for ISH signal between lots was calculated as 100% for 30 sections evaluated.



Diagnostic Sensitivity and Specificity

RNAscope ISH Probe High Risk HPV was compared to p16 immunohistochemistry (IHC) using 48 cases of oropharyngeal carcinoma. Specifically, serial sections from cases of tonsil, oropharynx, soft palate, back of the pharynx, and base of the tongue were stained with RNAscope ISH Probe High Risk HPV and control probes, as well as p16 IHC. One sample was excluded due to unacceptable RNA integrity as indicated by the UBC control probe. Comparative RNAscope and p16 results are summarized in the following table.

		p16 IHC	
		Positive	Negative
RNAscope ISH Probe High Risk HPV	Positive	24	0
	Negative	3	20

The positive percent agreement was 89% (24/27).

The negative percent agreement was 100% (20/20).

The agreement observed between RNAscope ISH Probe High Risk HPV and p16 IHC is consistent with the expectation for some p16 positive cases to be negative by ISH.⁴⁻⁶ The three discrepant cases may be the result of p16 activation through a non-HPV related mechanism. p16 is an indirect, surrogate marker for HPV; other mechanisms may upregulate p16 expression to provide a positive p16 IHC result in the absence of HPV. By contrast, ISH is a direct measure of HPV mRNA and therefore is not expected to produce positive results through non-HPV pathways.

LIMITATIONS

1. The method has been optimized for use on the Leica Biosystems BOND-III automated stainer using BOND RNAscope Brown Detection and ancillary BOND reagents. Users should be trained in the use of the BOND-III.
2. Modifications to the procedures are not recommended and could produce inaccurate results.
3. The assay has been validated with human oropharyngeal carcinoma FFPE tissue samples. Other sample types have not been evaluated.
4. Use of positive and negative control probes are necessary to properly interpret the assay. The positive control probe verifies RNA integrity in the sample. The negative control probe confirms that the sample is free of non-specific signal or interfering substances that would confound interpretation.
5. The assay does not genotype the high-risk HPV subtypes that are detected.
6. Tissue and cell staining is dependent upon the handling and processing of the tissue sample prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, false-positive, or false-negative results. Inconsistent results may be due to variations in fixation and embedding methods, or to inherent irregularities within the tissue sample.
7. Degradation of mRNA in the tissues over time can cause false-negative results. Stain the specimens within 3 weeks of mounting the tissues on slides when stored at room temperature (20-25 °C).



WARNINGS AND PRECAUTIONS

Formamide (≤ 50%).



Signal Word: Danger.

Hazard Statement(s):

H351 Suspected of causing cancer.

H360 May damage fertility or the unborn child.

H373 May cause damage to liver, kidney, and blood.

Precautionary Statement(s):

P201 Obtain special instructions before use.

P202 Do not handle until all safety precautions have been read and understood.

P281 Use personal protective equipment as required.

P308+P313 IF exposed or concerned: Get medical advice/attention.

P314 Get medical advice/attention if you feel unwell.

P405 Store locked up.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

1. Avoid direct contact with the probe. Use appropriate personal protective equipment (PPE) to prevent exposure to eyes, skin, and mucous membranes. If exposure occurs, wash liberally with water.
2. Probe contains material of animal origin. Consider all materials of human or animal origin as a risk for transmitting infection. Take adequate precautions for handling and ensuring proper disposal.
3. Microbial contamination can lead to inaccurate results.
4. Refer to the Safety Data Sheet for additional safety information available at www.bio-techne.com.
5. Consult relevant local authorities and regulations for proper disposal of the probe.

TROUBLESHOOTING

If expected results are not obtained with control tissue, repeat the test.

For troubleshooting information, contact Technical Support at support.acd@bio-techne.com.

SYMBOL DEFINITIONS

Catalog Number	Temperature limit	<i>In vitro</i> diagnostic medical device
Manufacturer	Batch code	European conformity
Use By date	Consult instructions for use	Authorized representative in the European Community
Serious health hazard		

INTELLECTUAL PROPERTY

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BOND is a trademark of Leica Biosystems.



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