RNAscope™ ISH Probe UBC (Positive Control)

REF **200460**

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

INTENDED USE

RNAscope ISH Probe UBC (Positive Control) is intended for use as a positive control in a RNAscope *in situ* hybridization (ISH) assay in formalin-fixed paraffin-embedded (FFPE) tissue specimens. The probe detects mRNA transcripts of the human ubiquitin C gene.

The probe is for use in clinical laboratories with BOND RNAscope Brown Detection on the automated Leica Biosystems BOND-III stainer. 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 UBC (Positive Control) contains oligonucleotide probes designed to hybridize to nucleic acid sequences of the human ubiquitin C gene in FFPE tissue sections. 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.[1,2] UBC signal should be observed in human tissues with sufficiently preserved RNA, suitable for the RNAscope assay.

REAGENT PROVIDED

RNAscope ISH Probe UBC (Positive Control) 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

- 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.

The staining procedure for RNAscope ISH Probe UBC (Positive Control) should be identical to that used for the target probe in an RNAscope assay. Run a positive probe control for every patient specimen using an adjacent serial section.

INTERPRETATION OF RESULTS

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

Positive signal appears as brown chromogenic dots, visible with a light microscope using a 20X or 40X objective lens.

Acceptable UBC staining is comprised of diffuse signal present across the tumor area, indicative of sufficient RNA integrity for interpretation of an RNAscope target probe.

If no staining, or only weak, focal staining is observed, the RNA may be compromised and the RNAscope target probe should not be interpreted.

PERFORMANCE CHARACTERISTICS

Analytical Specificity

Analytical specificity was tested using plasmids containing a portion of the UBC gene sequence that were spotted and cross-linked to glass slides. RNAscope ISH Probe UBC (Positive Control) was tested on the plasmid slide. The UBC plasmid was detected, while other, unrelated sequences were not detected.

Analytical Precision

The reproducibility of staining performance of RNAscope ISH Probe UBC (Positive Control) 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. Two sections of each of five FFPE tissues were stained on each day. The overall percent agreement for ISH signal between days was calculated as 100% for 50 sections evaluated.

Inter-instrument precision was conducted using one probe lot and three BOND-III instruments. Two sections of each of five FFPE tissues were stained on each instrument. The overall percent agreement for ISH signal between instruments was calculated as 100% for 30 sections evaluated.

Inter-lot precision was conducted using three probe lots. Two sections of each of five FFPE tissues were stained on each instrument. The overall percent agreement for ISH signal between lots was calculated as 100% for 30 sections evaluated.

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 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. 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.
6. False-negative results could be caused by degradation of mRNA in the tissues over time. Specimens should be stained within 3 weeks of mounting of 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

REF Catalog Number	Temperature limit	IVD *In vitro* diagnostic medical device
Manufacturer	LOT Batch code	CE European conformity
Use By date	Consult instructions for use	EC REP Authorized representative in the European Community
Serious health hazard		

INTELLECTUAL PROPERTY

REFERENCES

1. Wilkinson DG. The theory and practice of in situ hybridization. In: Wilkinson DG. (ed.) In situ Hybridization. A practical approach. 2nd Edition. New York: Oxford University Press, 1998, pp.18-20.
2. Wang F, Flanagan J, Su N, Wang LC, Bui S, Nielson A, Wu X, Vo HT, Ma XJ, Luo Y. RNAscope: a novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues. J Mol Diagn. 2012 Jan;14(1):22-9.

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