

## FAQs for RNAscope<sup>®</sup> FFPE Assay

### 1. How does RNAscope<sup>®</sup> FFPE assay compare with traditional RNA ISH assay?

RNAscope FFPE Assay offers single molecule detection sensitivity and superior specificity. The resolution of the signal is much higher and tissue morphology is much better preserved. The assay procedure is also much simpler and more robust and it can be completed within 6-7 hours.

### 2. How likely will my FFPE tissue specimens work with your RNAscope<sup>®</sup> FFPE Assay?

We have conducted extensive assay development to make sure that the RNAscope FFPE assay will work with a wide range of tissue types.

- If your tissue specimens are processed according to the [Specimen Preparation Guideline](#) in the [User Manual](#) and they are prepared within the last 3 years, you should expect successful staining for positive control probe using our assay procedure.
- If your specimens are prepared differently from our recommended procedure, RNAscope can still be used but may require assay optimization for some procedural steps. You may request a Specimen Assessment Service or conduct a Specimen Assessment Procedure by yourself to find out the probability of success in applying the standard RNAscope FFPE Assay to your specimen.
- Depending on the outcome of the Assessment, your specimen may work with the standard RNAscope FFPE Assay without any optimization, or ACD Technical Scientist may be helpful to advise you on how to best optimize certain assay conditions (e.g. diluting protease) to maximize the assay performance for your specimens.
- Under-fixed tissue specimens (e.g. fixed in 10% NBF for less than 12 hrs at RT) do not work well in the RNAscope FFPE Assay.

### 3. Is RNAscope<sup>®</sup> FFPE Assay difficult to run?

No. This is a much easier assay compared to a typical FISH assay procedure.

- The entire assay takes less than 6-7 hours.
- No flimsy cover slips or messy silicon gels to handle.
- No need for expensive instrument. Assay results are read with common bright-field microscope.
- The assay is so robust that most trained molecular biology technicians can obtain good results running it the first time out of box.
- You do need a HybEZ oven to conduct hybridization steps for best results with the manual assay.

**4. Our clinical tissue specimens are very precious and we would like to maximize the chance of success, can you run the RNAscope® FFPE Assay for us?**

Yes. You do have the opportunity to tap into our world-leading assay development expertise for *in situ* RNA detection. We can provide high quality service to run the RNAscope FFPE assay on your precious clinical samples. Please read [Research Service](#) for more information.

**5. What are the most critical factors affecting assay performance?**

Both temperature and humidity are critical to assay performance. The HybEZ oven is the only hybridization oven that we have extensively tested and validated. Other incubators/hybridization stations do not provide consistent results.

Protease digestion is also critical to assay performance. Under-digestion will result in lower signal and a ubiquitous background. Over-digestion will result in poor morphology and loss of RNA.

**6. Why should I run positive and negative control using the specimen I am testing?**

Retrospectively collected tissue specimens may not be prepared according to the [Specimen Preparation](#) guideline. Their RNA quality is also unknown. The UBC (POLR2A for RNAscope 2.0) positive control will help determine whether the RNA in the tissue specimen is of sufficient quality for detecting your RNA biomarker. The bacterial dapB negative control will help determine whether the tissue specimen is appropriately prepared. Only when the UBC positive control has a score of 3+/4+ (POLR2A 1+) and the DapB negative control has a score of 0/1+, you can confidently make a call on the expression of your target RNA in the tissue specimen.

**7. What are reference slides, and why should I run reference slides?**

For some popular markers, such as HER2 and EGFR2, we provide reference slide contains two FFPE cell lines with one expressing the target RNA at high level and another at low or minimal level. Since the FFPE cell lines are equivalently fixed and prepared as the FFPE tissue specimens, the slide serves as a positive control to ensure that your assay is appropriately run.

**8. Can I run the assay using smaller volume of reagents?**

The amount of assay reagents added into the slide are optimized and validated for a simple and robust RNAscope FFPE Assay. Using less than recommended volume will negatively impact the assay performance.

**9. Can I run the assay on a different type of FFPE tissue specimen?**

The RNAscope FFPE Assay kits have been developed and validated on human breast, colon, lung, prostate tumor specimens, as well as lymph node tissues. While it should work on other tissue types, optimal assay conditions (e.g. protease digestion time) need to be identified and validated. Please follow the procedure for [Assay Optimization Guideline](#) in our User Manual.

**10. What should I do if I don't know whether the FFPE tissue specimen is appropriately fixed and processed?**

Please follow the procedure for [Tissue Specimen Preparation and Assay Optimization Guideline](#) to determine whether your tissue specimen is appropriately fixed and processed and how to adjust assay conditions (e.g. diluting protease) to compensate.

**11. What's the impact of under-fixed or over-fixed FFPE tissue specimens on RNAscope<sup>®</sup> FFPE Assay?**

Under-fixed tissue specimen will result in protease over-digestion, which leads to loss of RNA and poor tissue morphology. Over-fixed tissue specimen will result in protease under-digestion, which leads to poor probe accessibility and low signal and signal/background ratio while maintaining excellent tissue morphology.

**12. Retrospective FFPE tissue specimens are known to have varying quality of degraded RNA. Can you detect RNA biomarkers in older FFPE tissue specimens?**

Yes. We have routinely detected RNA transcripts in three year old FFPE tissue specimens. Tissue specimens as old as 10 year old could also work although we don't have as much experience.

**13. How should I analyze RNAscope<sup>®</sup> FFPE Assay results?**

Analysis can be based on scoring based on staining intensity and amount of DAB or Fast Red precipitates, the percentage of stained cells, and the type of stained cells. Please follow the scoring guideline in the [Tissue Specimen Evaluation](#) in our User Manual.

**14. What's the major difference between scoring RNAscope<sup>®</sup> results and IHC results?**

RNAscope results are scored based on staining intensity and DAB or Fast Red precipitate visibility under 10x, 20x and 40x objective lens. For RNAscope<sup>®</sup> 2.0, scoring is based on the number of dot or dot clusters in each cell. IHC is typically scored under 10x objective lens based on intensity estimate.

## **15. What should I do if the results are not to my satisfaction?**

ACD is proud in itself for providing the highest quality product and service. Customer satisfaction is our number one priority. We will do our best to protect your investment in our product and will guarantee product performance. You should expect to receive satisfactory results if our assay procedure is followed correctly, appropriate assay controls are included, and your tissue specimens are prepared according to our [Specimen Preparation](#) guideline. If you encounter any product- or assay-related issue, please do call or email our [Technical Support](#) team. We will respond within 24 hours and will provide timely support to help resolve your issue. Every effort will be made to achieve complete customer satisfaction.