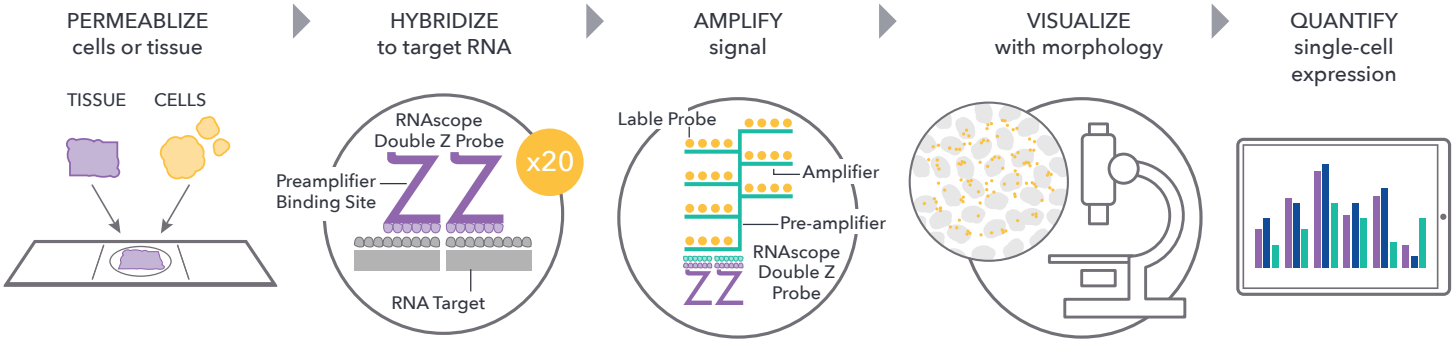
Accelerate COVID-19 research using the highly sensitive RNAscope™ *In Situ* Hybridization technology

The RNAscope technology:



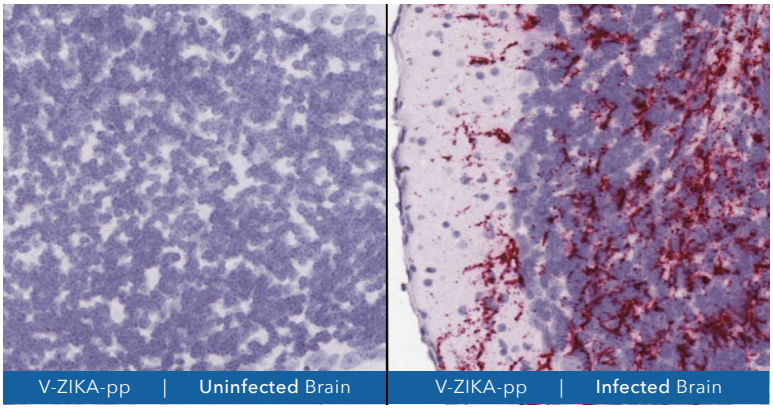
The RNAscope technology is a novel in situ hybridization method that is designed to detect target RNA in intact cells by using proprietary probes that simultaneously amplify target signal sequences while suppressing background noise from non-specific hybridization. This technology obviates the need for the costly and time-consuming development of specific antibodies for newly identified gene targets or pathogens such as the COVID-19 SARS-CoV-2 virus. Additionally, the RNAscope technology can be combined with immunohistochemistry (IHC) on the same slide for detection of RNA and protein simultaneously. With an ever-growing catalog of probes for over 25,000 targets in 300+ species and the option of made-to-order target probes, the RNAscope technology has become an essential technology for biomarker assessment, featured in over 2,700 publications across multiple different research areas including infectious disease, inflammation, neurobiology, cancer biology, and immuno-oncology.

Publications highlighting RNAscope in infectious disease research:

We have over 400 publications using the RNAscope technology for viral detection. Listed below are a few key publications on positive single-stranded RNA viruses.

1. Haagmans, B.L. et al., *An orthopoxvirus-based vaccine reduces virus excretion after MERS-CoV infection in dromedary camels.* Science, 2016. **351**(6268): p. 77-81.
2. Cha, R.H.Y., et al., *A Case Report of a Middle East Respiratory Syndrome Survivor with Kidney Biopsy Results.* J Korean Med Sci, 2016. **31**(4): p. 635-640.
3. Haagmans, B.L. et al., *Asymptomatic Middle East respiratory syndrome coronavirus infection in rabbits.* J Virol, 2015. **89**(11): p. 6131-6135.
4. Vergara-Alert, J.v.d.B., et al., *Livestock Susceptibility to Infection with Middle East Respiratory Syndrome Coronavirus.* Emerg Infect Dis, 2017. **23**(2): p. 232 - 240.
5. Bhatnagar, J.R., et al., *Zika Virus RNA Replication and Persistence in Brain and Placental Tissue.* Emerg Infect Dis, 2017. **23**(3): p. 405-415.

Detection of positive single-stranded RNA virus



Detection of ZIKA virus strain PRVABC59 in mouse tissues: The RNAscope probe detected positive staining for ZIKA viral RNA in the infected tissues but not in the uninfected tissues demonstrating the specificity of the RNAscope probes.

How can the RNAscope technology support COVID-19 research?

Applying the RNAscope technology to your COVID-19 research can provide new insights into the pathogenesis of SARS-CoV-2 and aid in vaccine and drug development efforts by helping researchers answer the following questions:

1. **Which tissue(s) does SARS-CoV-2 infect?**

   RNAscope assays allow for direct visualization of the virus and estimate the viral load in cells with morphological context. The **V-nCoV2019-S** probe detects the SARS-CoV-2 spike protein mRNA and does not detect other coronaviruses or host mRNA.

2. **Which cell type(s) does SARS-CoV-2 infect?**

   Using the RNAscope multiplex assays, **V-nCoV2019-S** probe can be combined with probes for the cellular receptor **ACE2** and proteases such as **TMPRSS2, Cathepsin B** and **Cathepsin L,** all of which facilitate viral entry into the host cells, for the visualization of infected cells. The single-molecule sensitivity of RNAscope is particularly well-suited for detecting low levels of ACE2 expression and identifying virus-targeted cell types.[1]

3. **What inflammatory response does the virus induce in infected patients?**

   Patients with severe COVID-19 infections suffer from acute respiratory distress syndrome (ARDS)[2] which is induced by the release of cytokines such as **IL-6, IL-1β, IL-10** and **TNFα** among others.[3] RNAscope assays can identify the cells secreting these inflammatory cytokines.

4. **In which tissues/cells does SARS-CoV-2 replicate?**

   The RNAscope assay can detect viral replication in tissues by using the **V-nCoV2019-S sense** probe targeting the antisense RNA strand produced during viral replication.

Listed below are probes and reagent kits for manual RNAscope assays.
Corresponding probes and reagent kits for automated assays are also available.

RNAscope tools available for COVID-19 research

CAT. NO.	PROBE	TARGET
848561	RNAscope® Probe - V-nCoV2019-S	Viral spike protein
845701	RNAscope® Probe - V-nCoV2019-S-sense	Spike protein sense strand
848151	RNAscope® Probe - Hs-ACE2	Host cell receptor
470341	RNAscope® Probe – Hs-TMPRSS2	Serine protease
602051	RNAscope® Probe – Hs-IL10	Cytokine
310371	RNAscope® Probe – Hs-IL6	Cytokine
310361	RNAscope® Probe – Hs-IL1B	Cytokine
310421	RNAscope® Probe – Hs-TNFA	Cytokine

CAT. NO.	ASSAY
322300	RNAscope® 2.5 HD Reagent Kit – BROWN
322350	RNAscope® 2.5 HD Reagent Kit – RED
322430	RNAscope® 2.5 HD Duplex Reagent Kit
323100	RNAscope® Multiplex Fluorescent Reagent Kit v2

Order probes: **https://bit.ly/rnascope-probes**

Learn more about RNAscope application for viral parthenogenesis research:
acdbio.com/science/applications/research-areas/infectious-diseases

References:

1. Hoffmann, M.; Kleine-Weber, H.; Schroeder, S.; et al. S. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell 2020, DOI: 10.1016/ j.cell.2020.02.052.
2. Z Xu, L Shi, Y Wang, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med 2020 , DOI: 10.1016/S2213-2600(20)30076-X.
3. Park WY, Goodman RB, Steinberg KP, et al. Cytokine balance in the lungs of patients with acute respiratory distress syndrome. Am J Respir Crit Care Med 2001, DOI: 10.1164/ajrccm.164.10.2104013.

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