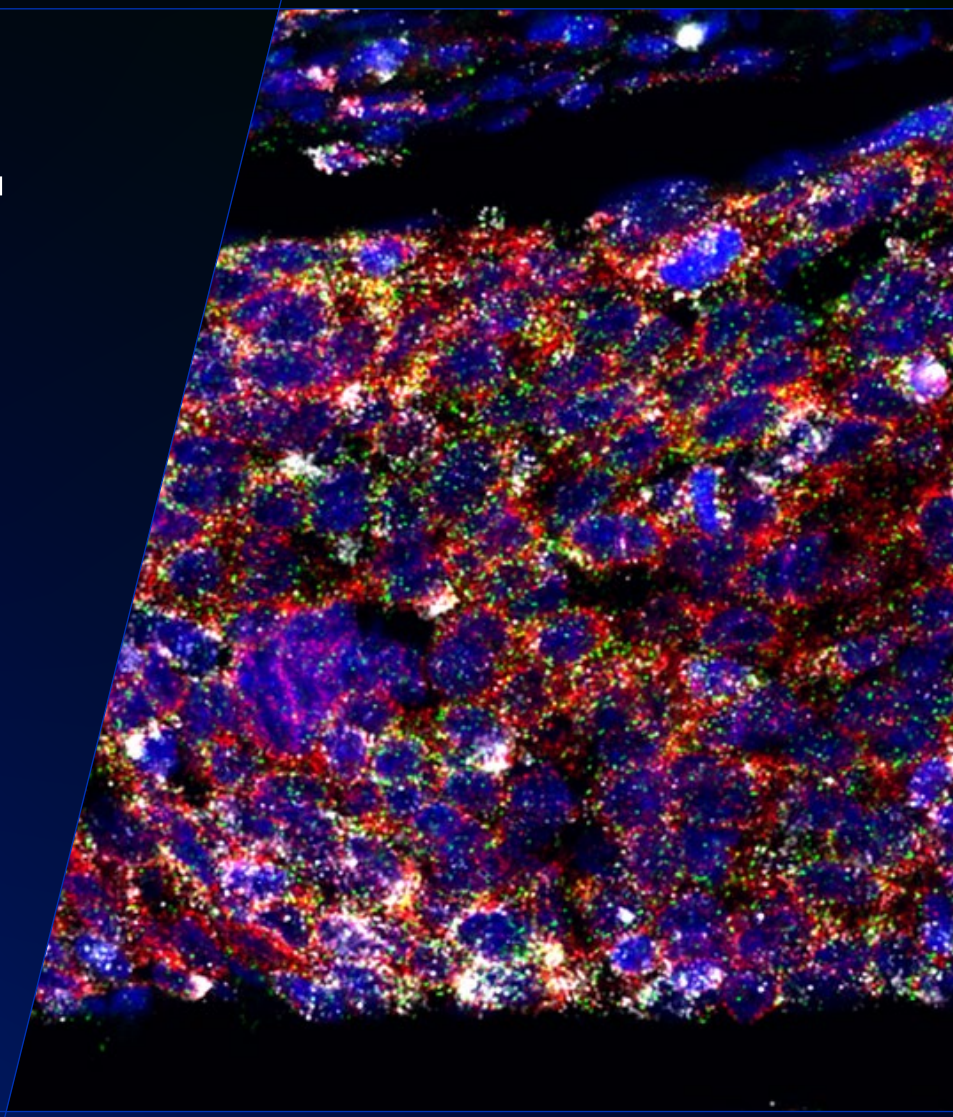
bio-techne
spatial™

RNAscope™ HiPlex v2 Assay



Enhancing Your Spatial Genomics Story

Introducing HiPlex Assay

RNAscope™ HiPlex v2 assay is an expansion of the RNAscope HiPlex assay, an advanced *in situ* hybridization (ISH) tool to understand vital gene expression patterns at single cell resolution.

The new assay generates precise gene expression data, allowing researchers to conduct comprehensive spatial studies from 12 targets in FFPE samples and up to 48 targets in Fresh and Fixed Frozen sample types.

The assay enables direct visualization of the transcript by eliminating the need for difficult bioinformatics methods to analyze and interpret scRNA-seq data. It also provides accurate and reliable data with a simple workflow, making it the assay of choice for translational research and biomarker discovery.

This highly sensitive and multi-plexing assay can be performed on commonly available laboratory equipment without the need to invest in expensive capital equipment.

Assay Features

- Higher Plexing Capability
- All Tissue Types - FFPE, Fresh and Fixed Frozen
- Single Molecule Detection Capability
- True Single Cell Resolution
- No Degradation Bias
- Enzyme Free Signal Amplification
- Effortless Probe Design
- Novel Image Registration Software

Spatial transcriptomics enabled by direct visualization of RNA molecules in intact samples holds great promise in resolving heterogeneous tissues at single cell and sub-cellular resolution. Identifying genes that are differentially expressed in distinct cell populations provide insights into cellular organization and function of diverse cell types in healthy and disease states.

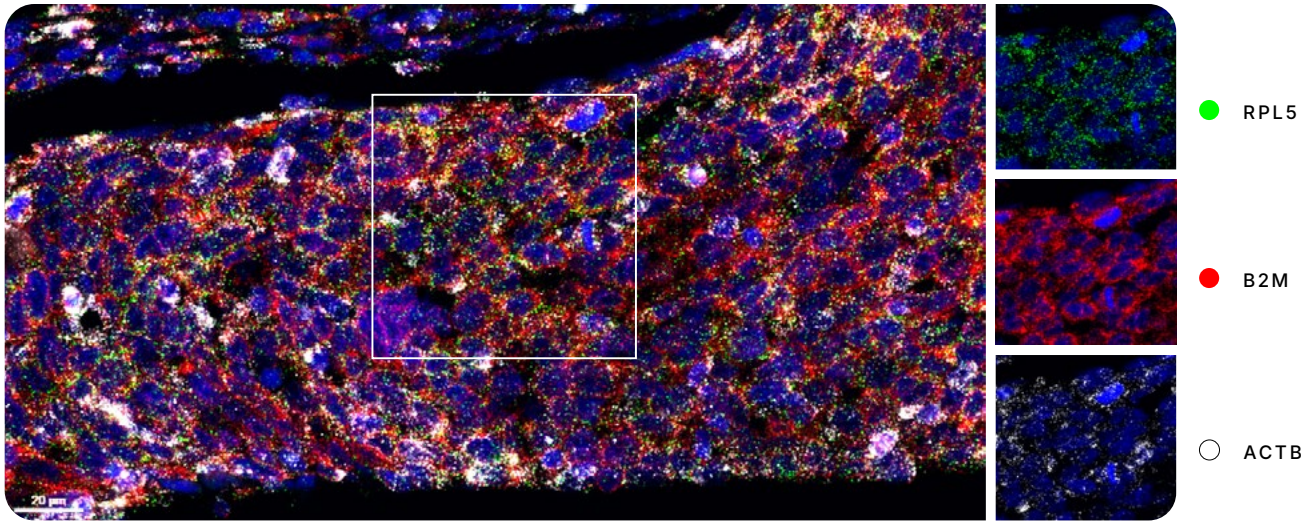


Figure 1. RNAscope™ HiPlex assay on human FFPE cervical cancer tissue, using Alexa 488, DL550 and DL650 fluorophores for the detection of HiPlex12 positive control probes RPL5, B2M, and ACTB.

Assay Workflow

The HiPlex assay offers a powerful and efficient plexing tool for validation and visualization of multiple targets with a vast catalog of RNAscope probes and rapid turn-around-time for custom probes for any target. The advanced capabilities of this assay increases confidence in higher plexing data platforms that traditionally rely on grind and bind or enzymatic amplification based approaches.

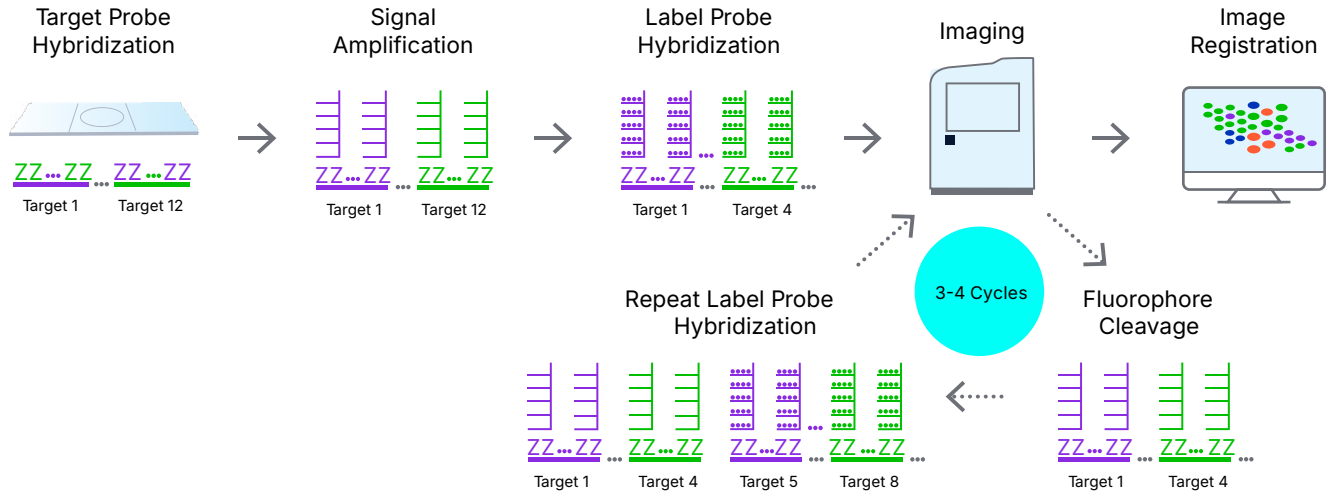


Figure 2. RNAscope HiPlex assay provides signal amplification for simultaneous visualization of up to 12 RNA targets in FFPE and up to 48 targets in fresh and fixed frozen samples by performing iterative fluorescent imaging rounds.

Enabling Multitude of Applications

RNAscope™ HiPlex assay has applications across multiple research areas including novel applications such as complex tissue profiling and single cell validation.

Complex Tissue Profiling

Complex tissues are comprised of multiple cell types with dynamic interactions that are governed by their spatial organization. The RNAscope HiPlex assay can help establish gene signatures within tissues and uncover critical pathways implicated in disease pathology.

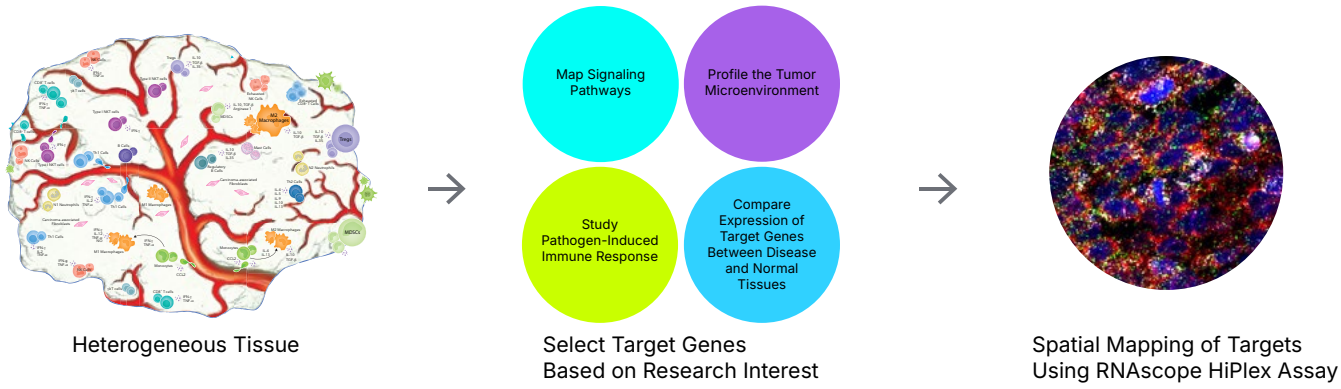


Figure 3. Illustration of complex tissue profiling workflow using RNAscope HiPlex assay to resolve cellular heterogeneity by spatially validating targets of interest within intact tissue samples across various research areas.

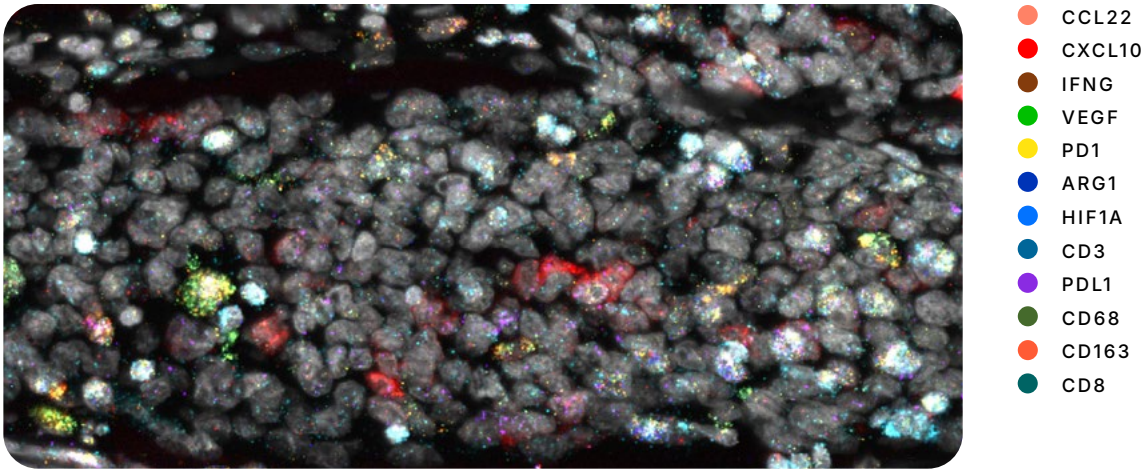


Figure 4. Detecting immune cells, chemokines, cytokines and cancer cell markers by spatially profiling the cervical cancer TME.

Single Cell Transcriptomic Validation

The RNAscope™ HiPlex assay provides a multiplexing solution for orthogonal confirmation and spatial mapping of scRNA-seq data by enabling simultaneous visualization of up to 12 target genes.

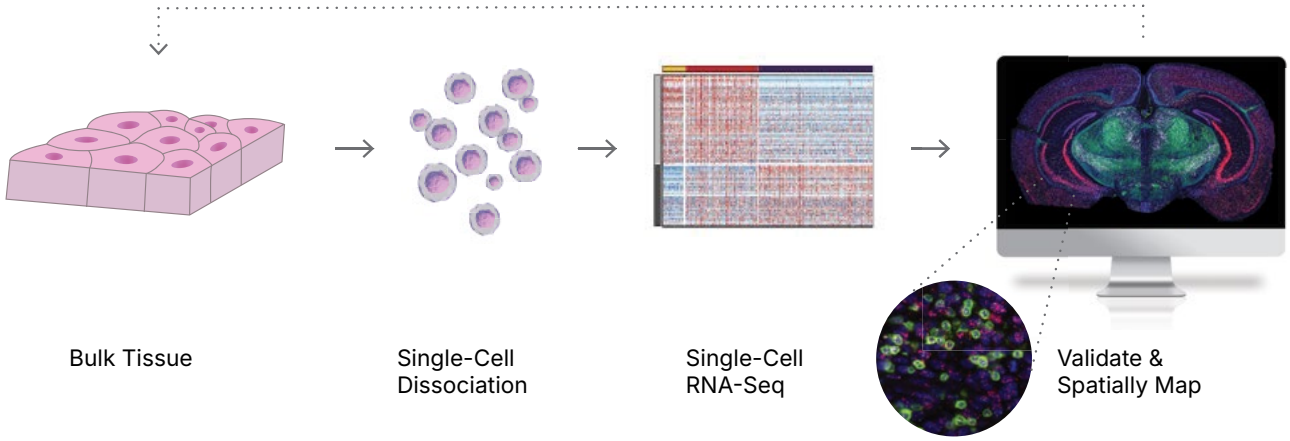


Figure 5. Incorporation of spatial validation into single cell sequencing based transcriptomic workflows with the RNAscope ISH technology.

RNAscope HiPlex imaging of Drd1a and Drd2 medium spiny neuronal (MSN) subtypes

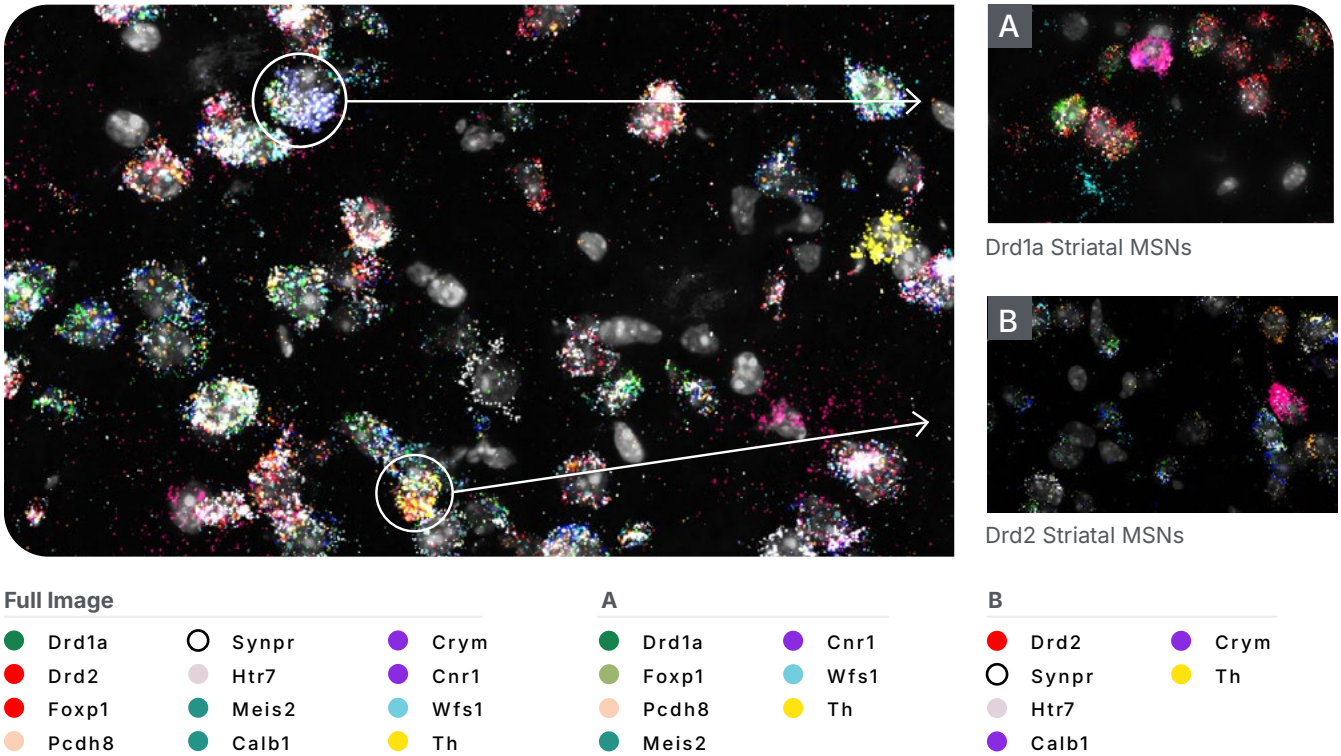
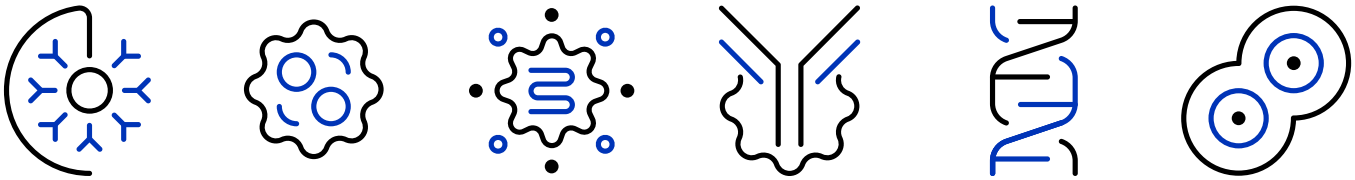


Figure 6. **Simultaneous visualization and characterization of the D1 and D2 subtypes in the mouse striatum with the RNAscope HiPlex assay.** The D1 subtypes are characterzied by Drd1a, Foxp1, Pcdh8, Meis2, Dner, Cnr1, Crym, and Wfs1 **(A)**, whereas the D2 subtypes are characterized by Drd2, Calb1, Cartpt, Synpr, Htr7, Crym, Cnr1, and Th. **(B)** Insets show cells expressing markers indicative of the major D1 **(A)** and D2 **(B)** subtypes. Validation of striatal neuronal subpopulations identified by scRNAseq as described in Gokce et al., Cell Rep, 2016.

Product Information and Resources

Research areas enabled by RNAscope™ HiPlex applications:



Neuroscience | **Oncology** | **Immunology/ Inflammation** | **Immuno-oncology** | **Translational Research** | **Stem Cell Research**

Assay Checklist

- **HiPlex Reagents Kit** Pretreatment Kit, Detection Kit, and Wash Buffer and Cleaving Reagent Kits
- **HiPlex Control Probes** Positive and Negative Control probes
- **HiPlex Probe Diluent** Probe Preparation Reagents
- **HiPlex Control Slides** Recommended set of control slides
- **RNAscope HiPlex Image Registration Software** Image Registration Software to register and merge the images acquired after performing the HiPlex Assay
- **Hydrophobic Barrier Pen** Heat-stable water-repellent barrier to keeps reagents localized on tissue sections

Start With Intro-Packs

Intro-packs include all the components needed to run the assay - HiPlex Reagent Kit, HiPlex Positive and Negative Control Probes, HiPlex Probe Diluent, HiPlex Control Slides, HiPlex Image Registration Software and Hydrophobic Barrier Pen.

Product Name	Product Number
RNAscope Intro Pack for HiPlex12 Reagents Kit (488, 550, 650, 750) - Mouse	324440
RNAscope Intro Pack for HiPlex12 Reagents Kit (488, 550, 650, 750) - Rat	324441
RNAscope Intro Pack for HiPlex12 Reagents Kit (488, 550, 650, 750) – Human	324442
RNAscope Intro Pack for HiPlex12 Reagents Kit (488, 550, 650) - Mouse	324443
RNAscope Intro Pack for HiPlex12 Reagents Kit (488, 550, 650) - Rat	324444
RNAscope Intro Pack for HiPlex12 Reagents Kit (488, 550, 650) - Human	324445

Leveraging our HiPlex assay chemistry, RNAscope HiPlexUP reagent enables expansion of our multiplexing capability of the RNAscope HiPlex assay to 48 targets on Fresh and Fixed Frozen sample types.

Product Name	Product Number
RNAscope HiPlexUp Reagent	324190

Additional Resources

- **HiPlex webpage –** bio-techne.com/hiplex
- **Single cell webpage -** bio-techne.com/applications/spatial-biology/rnascope-spatial-transcriptomics
- **Webinars -** bio-techne.com/resources/webinars/view-spatial-webinars
- **Tech support resources -** bio-techne.com/reagents/rnascope-ish-technology/get-started

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