

**Spatial Biology**

# **Visualize RNA biomarkers with exceptional sensitivity**

RNAscope™ Multiplex Fluorescent V2  
assay with New TSA Vivid™ Dyes

**biotechne®**

# RNAscope™ Multiplex Fluorescent V2 assay with New TSA Vivid™ Dyes for spatial interrogation of complex tissues

The RNAscope™ Multiplex Fluorescent V2 assays provide high sensitivity and allow single-molecule detection of up to 4 RNA targets simultaneously. With the new TSA Vivid™ dyes that are now part of the assay, we can visualize brighter signals with the same patented signal amplification technology.

Fluorescent tyramides (TSA) are a critical reagent for the RNAscope Multiplex Fluorescent V2 Assay. The TSA Vivid™ fluorophores with optimized TSA chemistry offer an effective way to enhance signal intensity and detect low-abundance targets in spatial biology applications. They are suitable for multiplexing and can be combined with DAPI counterstaining.

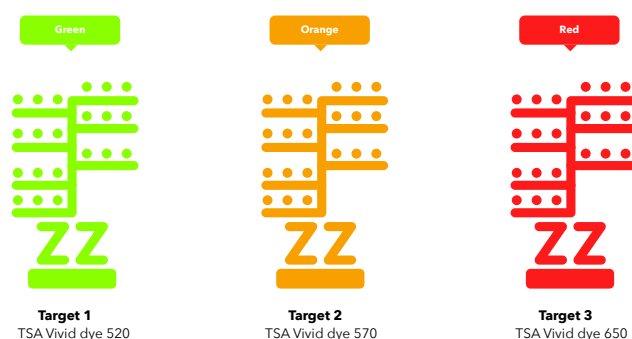
The new RNAscope Multiplex Fluorescent V2 assays combined with Vivid dyes can be utilized for a wide range of applications such as:

- Identifying immune and tumor cell interactions in tumor microenvironment.
- Detecting pathogens and host cell markers
- Characterizing neuronal sub-populations in functional regions of the brain
- Identifying cellular source of secreted proteins such as chemokines and cytokines.
- RNA-protein co-detection

## KEY FEATURES

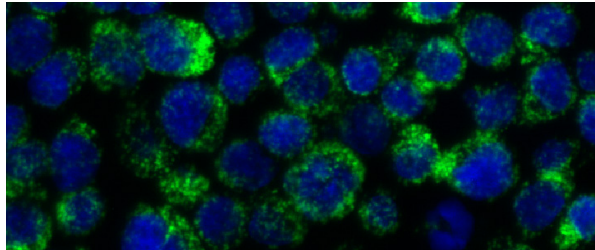
- Simultaneous detection of 4 RNA or 3 RNA + 1 protein targets in FFPE and fixed or fresh frozen tissues.
- The Multiplex Fluorescent V2 assay is enabled with Tyramide Signal Amplification (TSA) detection chemistry which improves sensitivity to detect low abundance targets.
- Re-engineered Tyramide Vivid dyes boost signal intensity and increase ease of target visualization.
- Vivid dyes are the best performing tyramide reagents for the RNAscope Multiplex Fluorescent V2 assay.

## Probe Hybridization, Signal Amplification and Detection using RNAscope Multiplex Fluorescent V2 Assay with TSA Vivid Dyes

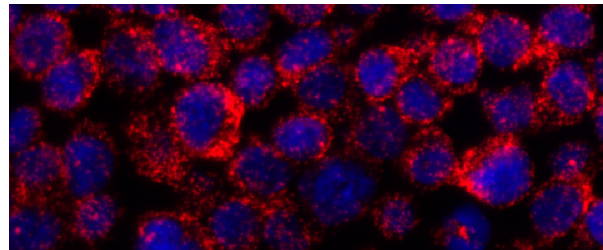


# Examples of multiplex RNA detection and RNA-protein co-detection using the RNAscope Multiplex Fluorescent V2 Assay with TSA Vivid dyes in different tissue types.

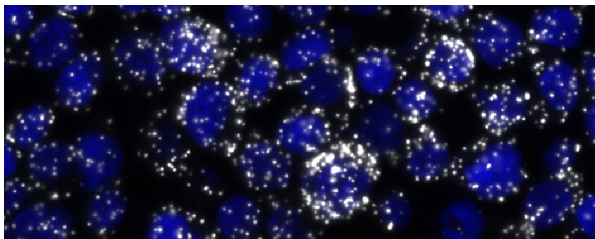
## Performance of TSA Vivid Dyes Detecting RNAscope Positive Control Targets in HeLa Cells



*POLR2A*-TSA Vivid 520



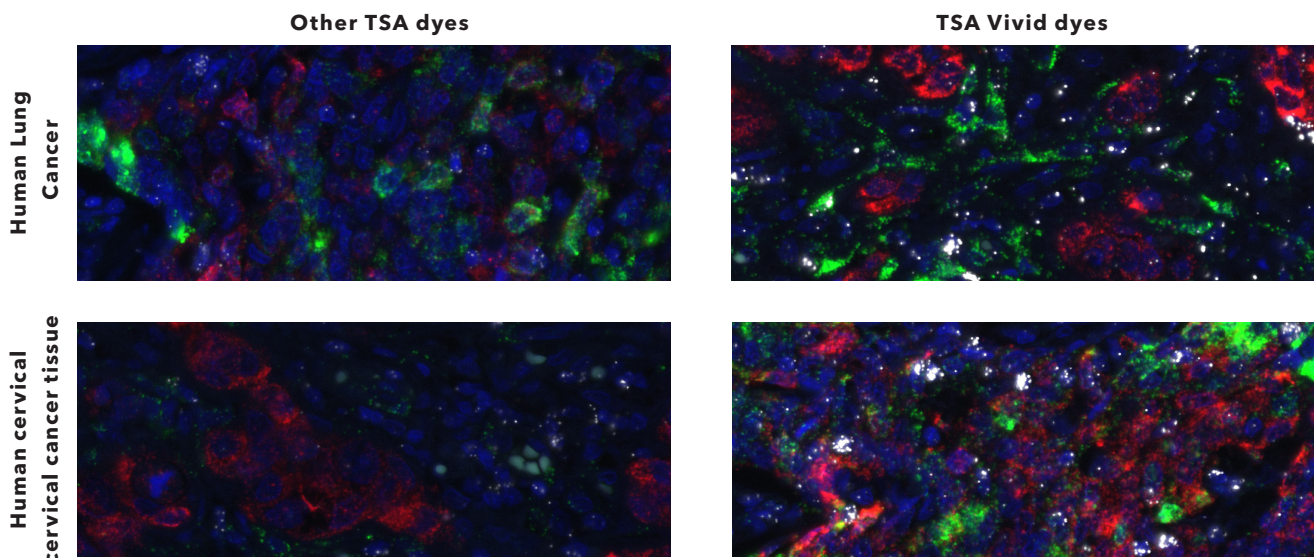
*PPIB*-TSA Vivid 570



*UBC*-TSA Vivid 650

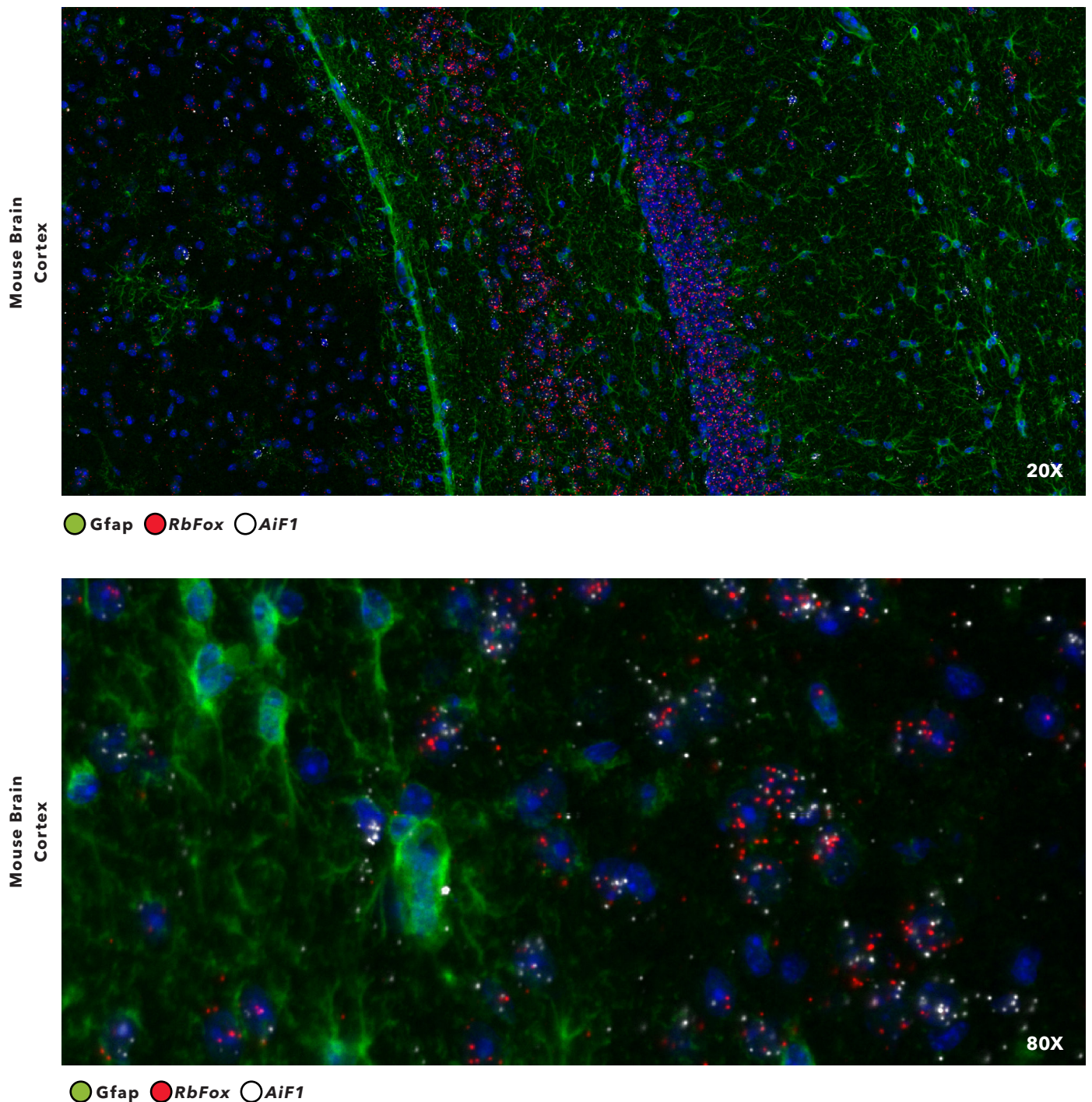
**Figure 1. RNAscope Multiplex Fluorescent V2 Assay with new TSA Vivid dyes.** RNAscope Multiplex Fluorescent v2 Assay on HeLa cells with TSA Vivid dyes detecting *POLR2A* (520), *PPIB* (570) and *UBC* (650). All dyes were used at 1:1500 dilution. Nuclei are counter-stained with DAPI.

## Comparing TSA Vivid Dyes Performance to other Popular TSA Dyes using the RNAscope Multiplex Fluorescent V2 Assay



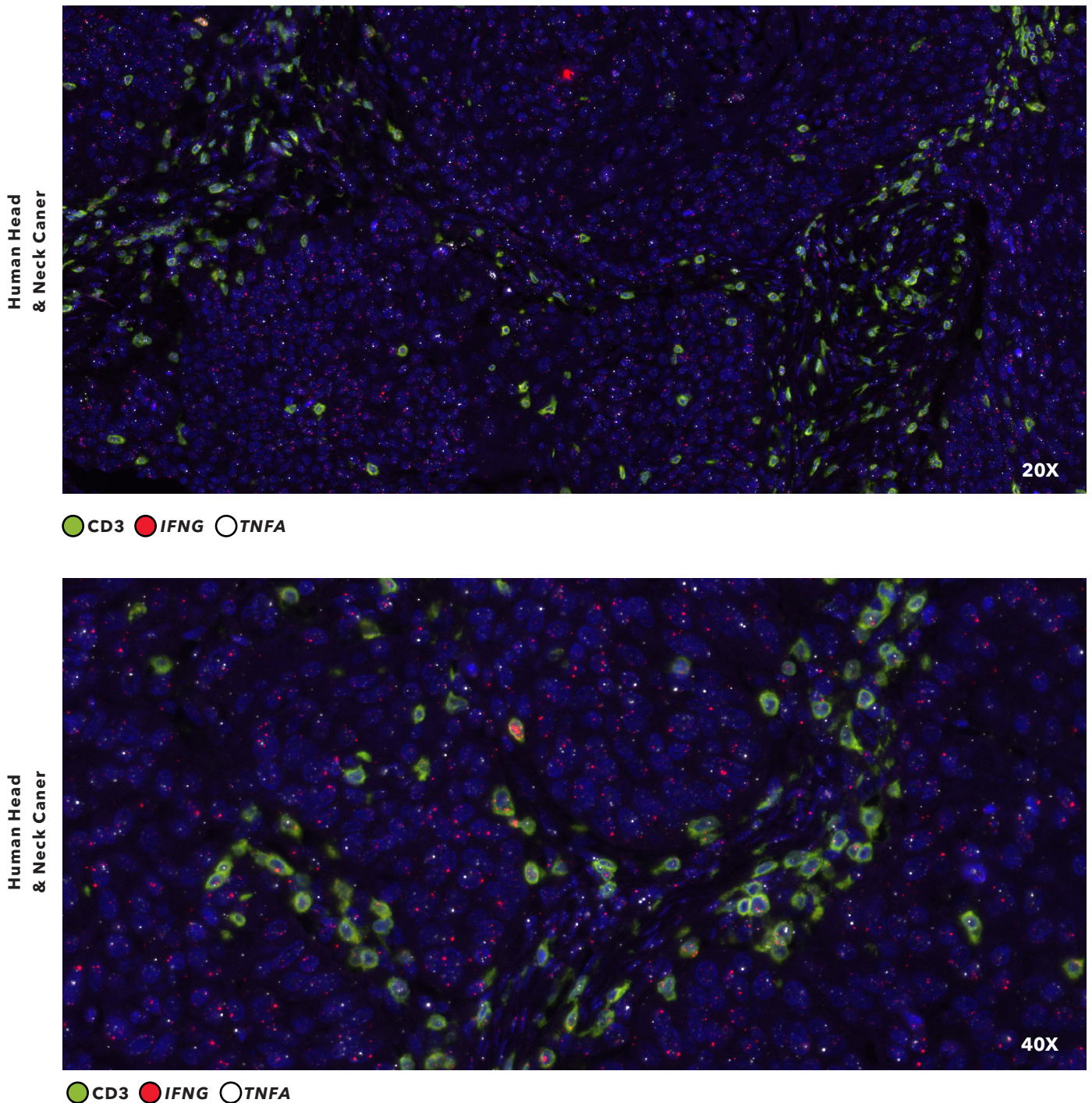
**Figure 2. 3-Plex immune-oncology marker detection on human cancer tissue using RNAscope Multiplex Fluorescent assay V2 with Vivid dyes.** Dilution was 1:1500 for all dyes *IDO-1*; FITC (520nm), *KRT-19*; Cy3 (570nm), *PD-1*; Cy5 (650nm). Much shorter exposure times were used for Toctris due to higher intensity of signal. Nuclei are counter-stained with DAPI.

RNA-Protein Co-detection to Visualize  
Cell-Type Specific Markers in the Brain using the  
RNAscope Multiplex V2 Assay with TSA Vivid Dyes.



**Figure 3. ISH-IF Integrated Co-detection of GFAP protein with *RbFox3* and *Aif1* RNA using RNAscope Multiplex Fluorescence V2 assay with Vivid dyes in fresh frozen mouse brain.** P56 sagittal mouse brain sections stained with GFAP antibody (green 520) along with *RbFox3* (red 570) and *Aif1* (white 650) RNA markers in distinct regions of the brain. Images depicted at (A) 20x and (B) 80x. Nuclei are counter-stained with DAPI.

# RNA-Protein Co-detection to Visualize Immune Cell Markers and Cytokines in a Tumor using the RNAscope Multiplex V2 Assay with TSA Vivid Dyes



**Figure 4. ISH-IF Integrated Co-detection of CD3 protein with *IFNG* and *TNFA* RNA using the RNAscope Multiplex Fluorescence V2 assay with Vivid dyes in FFPE Head and Neck cancer tumor.** CD3 antibody (green 520) with *IFNG* (red 570) and *TNFA* (white 650) RNA markers. Images depicted at (A) 20x and (B) 40x respectively. Nuclei are counter-stained with DAPI.

# Product Information:

Reagent Kits		
Component	Catalog Number	Assay Compatibility
RNAscope™ Multiplex Fluorescent Reagent Kit v2 with TSA Vivid™ Dyes	323270	For Manual workflow
RNAscope™ LS Multiplex Fluorescent Reagent Kit with TSA Vivid™ Dyes	323275	For Leica Bond Rx workflow

TSA Vivid Dyes		
Component	Catalog Number	Assay Compatibility
TSA Vivid™ Fluorophore 520	323271	RNAscope Multiplex Fluorescent V2 assay
TSA Vivid™ Fluorophore 570	323272	RNAscope Multiplex Fluorescent V2 assay
TSA Vivid™ Fluorophore 650	323273	RNAscope Multiplex Fluorescent V2 assay



Universal for virtually any gene and species



Specific and sensitive with performance reliability



Over 40,000 unique pre-designed probes available to select from



2-week turn around time for custom designs



Ease of use with ready to use formulations

# Largest catalog for pre-designed RNA probes

BioTechne can make probes for any RNA on demand. Using proprietary ACD RNAscope™ Probe Design Pipeline, we provide double “Z” oligo probes designed to hybridize to your specific RNA target of interest. We can design *in situ* hybridization RNA probes for virtually ANY gene in ANY genome for visualization in ANY tissue or Cells.

## Made-to-Order RNAscope Target Probe can Accommodate your Specific Needs:

If your gene of interest is not listed in our catalog, ACD can design and manufacture new *in situ* hybridization probes for you, we term these “RNAscope™ Made-to-Order Target Probes”



- All transcript variants: The design aims to detect all transcript variants in its target gene. For mRNA, this includes both the target coding (CDS) and/or non-coding UTR regions.
- Transcript variants (splice variants): Transcript variant-specific probes can be designed for regions with at least 300 unique.
- Specific regions within a single transcript variant: If your research requires a probe targeting only a specific region of the target RNA.
- Gene families: A standard design will target a single member of a gene family. Even among highly conserved gene families, it is typically possible to identify a region of suitable length (> 300 bases) that has less than 85% homology to any other gene, enabling single-target specificity of the probe.
- Multi-gene pools: ACD can design a set of probes that can be pooled together for studies where detection of any one of a set of markers is a relevant strategy.

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