High-throughput automated protease-free workflows for RNA-protein co-detection using RNAscope™ technology

Qi Shen, Renzo Adilardi, Emerald Doolittle, Rose Delvillar, John Donaldson, Li-Chong Wang, Aman Tyagi, **Debia Wakhloo**, Anushka Dikshit, Henry Lamparski, Maithreyan Srinivasan.

Advanced Cell Diagnostics, a Bio-Techne brand, Newark, California, USA

Introduction

RNA biomarker detection is key in assessing disease prognosis, predicting therapeutic response and evaluating therapeutic efficacy in cancer. Additionally, it is equally important to identify cell types expressing target RNA biomarkers using target marker antibodies. For example, studying tumor-infiltrating immune cells within the tumor can be vital for predicting response and determining therapeutic efficacy. This necessitates the use of spatial modalities that allow RNA and protein detection on the same tissue. RNAscope is an established leader in RNA biomarker detection with high performing high throughput automated ISH assays. To enable simultaneous detection of protein , we have developed a protease-free workflow for RNA-Protein co-detection by in situ hybridization followed by immunohistochemistry (ISH-IHC).

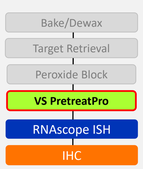
Here we demonstrate the protease-free workflow on the BOND Rx using the Chromogenic red assay by combining *PCA3* RNA with CD3 and CD8 proteins in prostate cancer tissue. Similarly, we also demonstrate the protease-free workflow on Roche's Ventana Discovery Ultra platform using the *IgK* RNA and CD20 protein in tonsil tissue and *VEGF* RNA and CD31 protein in lung cancer.

The protease-free workflow significantly improved the performance of protease-sensitive antibodies such as CD8, CD31 and CD20 while maintaining the RNA signal quality and preserving tissue morphology. *PCA3* is a key RNA biomarker in prostate cancer prognosis and demonstrated proximity to T cells identified by CD3 protein. Similarly, IgK RNA is a prognostic marker for B-cell lymphomas and demonstrated co-expression with B cells identified by CD20. Neo-angiogenesis in lung tumor was indicated by niche areas with high CD31 protein expression and high *VEGF* RNA expression.

Method

Here, we demonstrate the new protease-free workflows using our **RNAscope™ 2.5 LS assays** and **RNAscope™ VS Universal assays.**

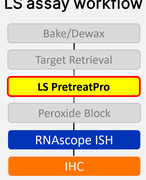
RNAscope VS Universal assay workflow



Targets and tissues

RNA	Protein	Tissue
IgK	CD20	Tonsil
VEGFA	CD31	Lung cancer
PD-L1	PD1	Tonsil

RNAscope 2.5 LS assay workflow



Targets and tissues

RNA	Protein	Tissue
DapB	CD8	Prostate cancer
PCA3	CD8	Prostate cancer
PCA3	CD3	Prostate cancer

Results

RNAscope protease-free workflow rescues signal for a protease-sensitive antibody with the RNAscope VS Universal assay.

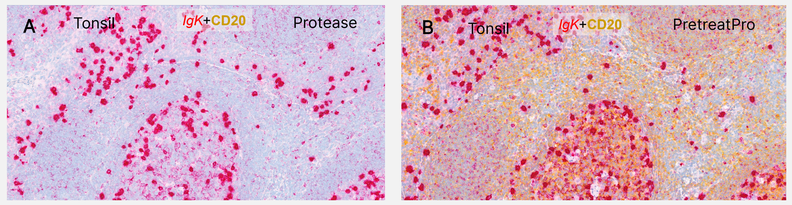


Figure 1: Visualizing B cell markers with protease and PretreatPro reagents. (A) CD20 protein signal was significantly diminished with protease pretreatment when combined with *IgK* RNA (red) detection, (B) the new VS PretreatPro pretreatment reagent enabled strong CD20 signal (yellow) detection in combination with *IgK* RNA (red).

The new protease-free workflow allows detection of RNA-protein biomarkers simultaneously without compromising on signal quality with the RNAscope VS Universal assays.

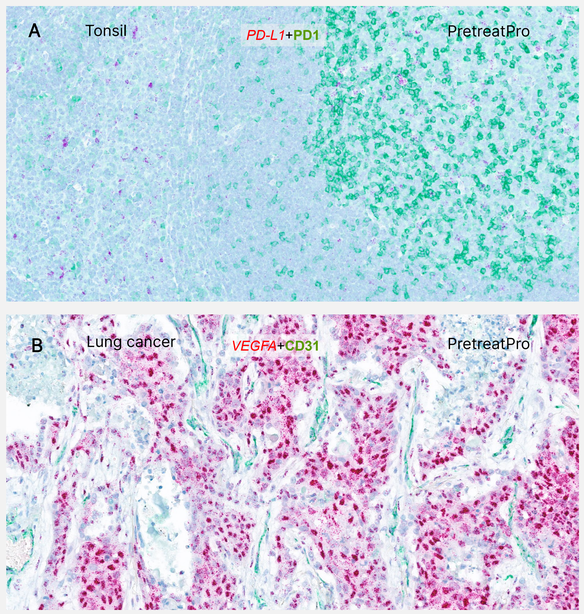
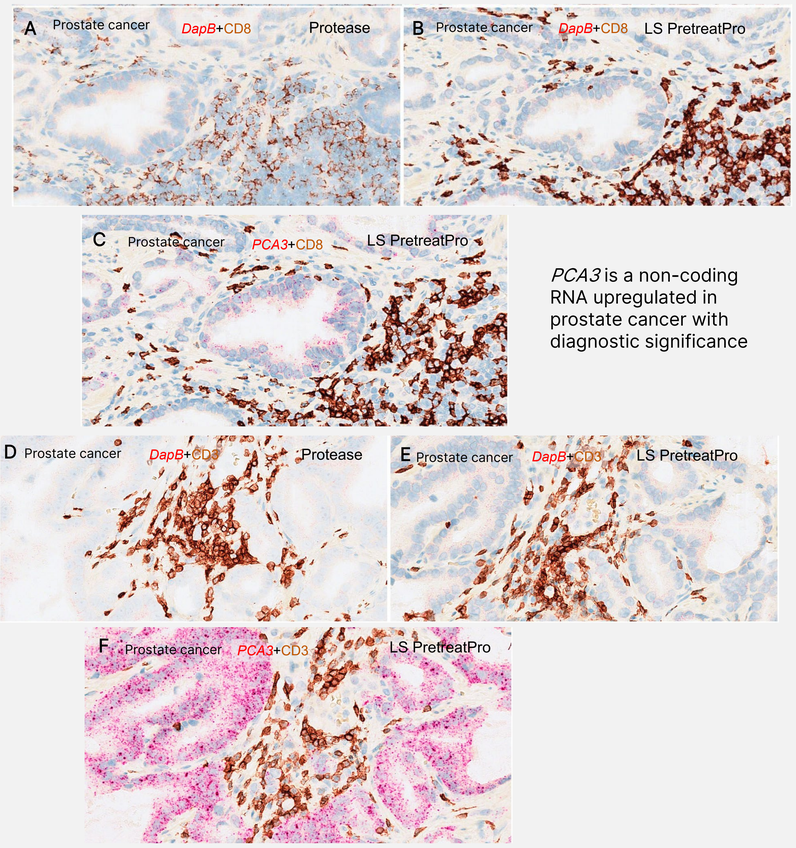


Figure 2: ISH-IHC using the protease-free workflow. (A) PD1 protein (green) was detected in combination with *PD-L1* RNA (purple) in tonsil tissue. (B) CD31 protein (green) was detected in combination with *VEGFA* RNA (purple) in lung cancer tissue.

RNAscope protease-free workflow rescues signal for a protease-sensitive antibody with the RNAscope 2.5 LS assay.



PCA3 is a non-coding RNA upregulated in prostate cancer with diagnostic significance

Figure 3: Protease-free workflow with a protease-sensitive and a protease non-sensitive antibody.(A) CD8 protein signal (brown)was significantly diminished with protease treatment (B) and was rescued by the new LS PretreatPro reagent. (C) CD8 protein was successfully detected in combination with *PCA3* RNA (red) in prostate cancer tissue. (D-E)Protease non-sensitive antibody CD3 showed comparable signal between protease and PretreatPro conditions. (F) CD3 protein detected in combination with *PCA3* RNA .

Summary

- The VS PretreatPro and LS PretreatPro reagents successfully enable detection of protease-sensitive targets in combination with RNA markers.
- These new workflows allow precise, high throughout detection of RNA and protein biomarkers simultaneously with optimal signal detection and morphology preservation.
- Co-detection of RNA and protein markers is vital to address key biological questions for developing next generation diagnostic and therapeutic strategies.