## Biomarker Datasheet

# Human TROP2 OmniVUE™ Biomarker

### Product Overview

TROP2 (Trophoblast cell surface antigen 2) is a transmembrane glycoprotein that plays a critical role in cell proliferation and signal transduction. It is highly expressed in most human cancers but hardly expresses in normal tissues, making it an ideal target for cancer therapy[1]. TROP-2 overexpression is associated with aggressive tumor behavior and poor prognosis[2], particularly in cancers such as breast, prostate[3], and colorectal cancer[4]. Its role as a target in antibody-drug conjugate therapies is currently under active investigation.

### Overview

| Target | Other names | Isotype | Primary cell type | Subcellular location | Indications of interest |
|---|---|---|---|---|---|
| **TROP-2** | GA733-1<br>M1S1<br>TACSTD2<br>EGP-1 | Rabbit IgG | Epithelial cells, highly expressed in cancerous epithelial tissues | Plasma Membrane | Breast CA<br>Lung CA<br>Colorectal CA |

*Clone available upon request

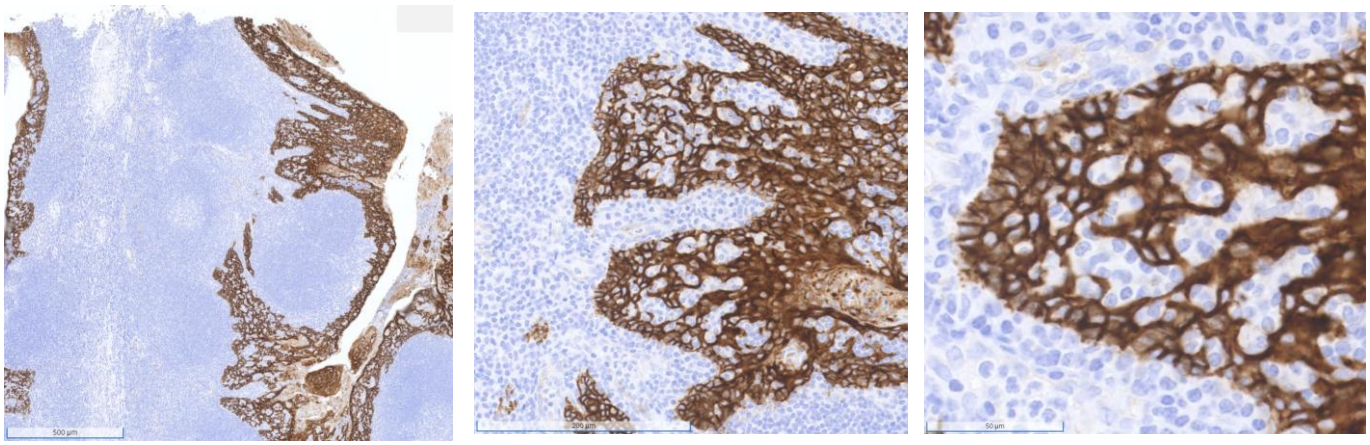
### Quality Control

Each lot of Antibody-barcode conjugate reagent is tested on the appropriate positive control tissue and reviewed by Ultivue's pathologists and image analysis experts to ensure expected staining pattern and positive signal intensity, through qualitative as well as quantitative analysis. Lot-to-lot consistency is evaluated and strictly maintained through quantitative comparison of a new lot of reagent with the predicate (previous lot) with an accepted variability of ±20% for positive signal intensity, which is at par with the current standards practiced in pathology.

## Predicate Comparison

Serial sections of a tonsil positive control and a colorectal cancer (CRC) tissue were stained with traditional chromogenic DAB using unconjugated antibodies and with the InSituPlex® (ISP) monoplex assay to demonstrate staining concordance between the two different modalities.

**TROP-2 Protein Expression in Tonsil tissue Using DAB**



**TROP-2 Protein Expression in Tonsil Tissue Using InSituPlex® (ISP) monoplex assay**

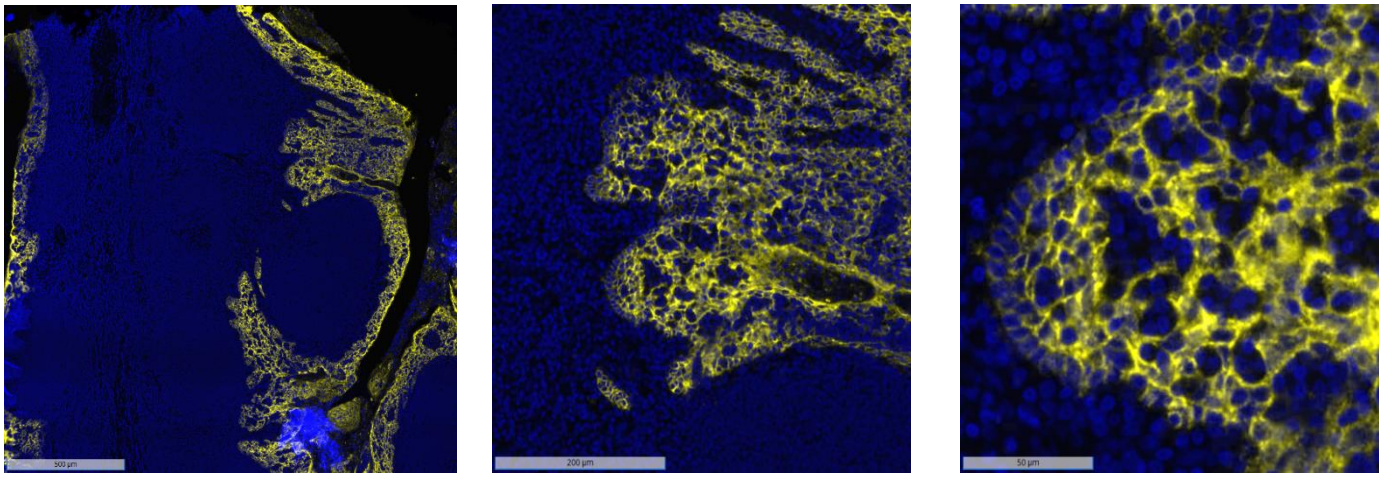
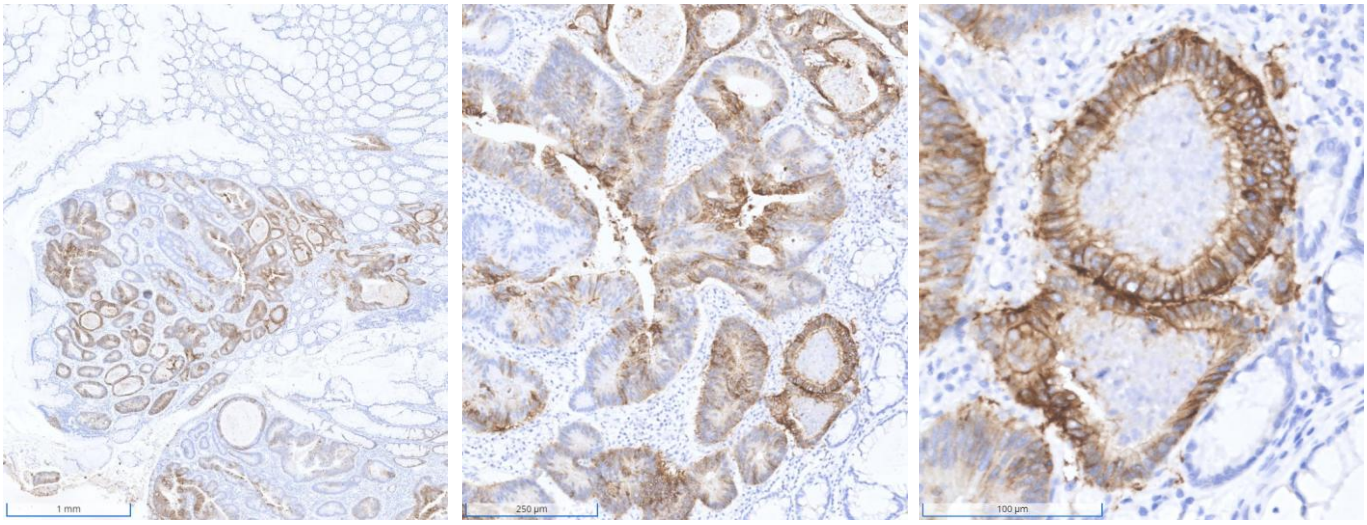


**Figure 1:** Comparison of DAB and InSituPlex® monoplex assay in Tonsil tissue. Chromogenic DAB (top panel), fluorescent ISP staining (bottom panel).

**TROP-2 Protein Expression in Colorectal Cancer (CRC) Tissue using DAB**




**TROP-2 Protein Expression in Colorectal Cancer (CRC Tissue Using InSituPlex® (ISP) monoplex assay**

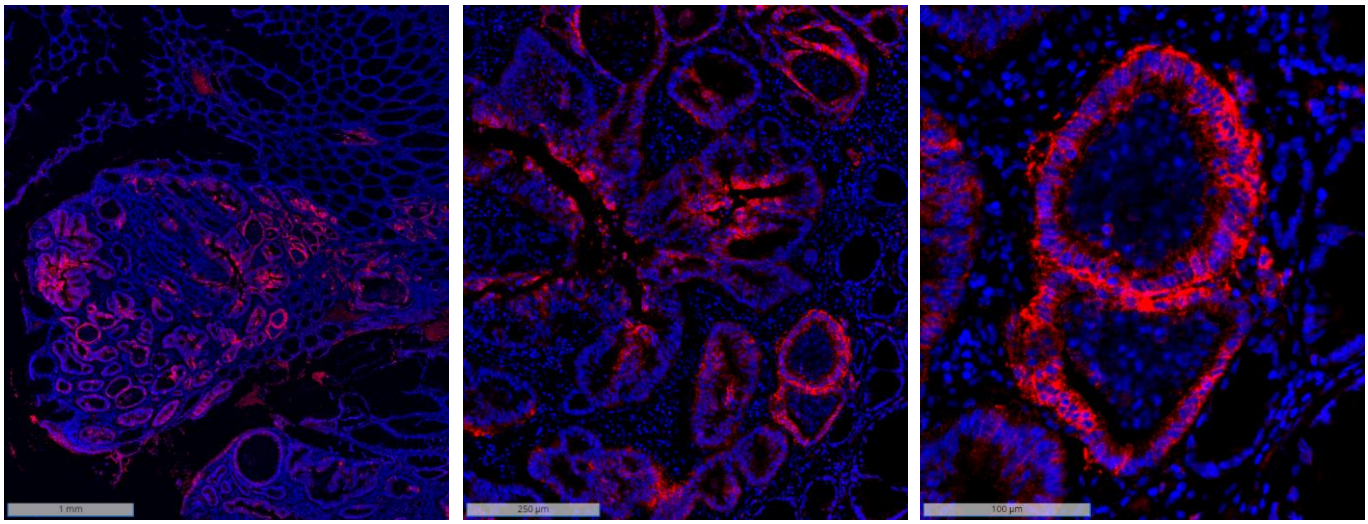



**Figure 2:** Comparison of DAB and InSituPlex® monoplex assay in CRC tissue. Chromogenic DAB (top panel), fluorescent ISP staining (bottom panel).

## Assay Precision Testing

Precision of the InSituPlex® monoplex assay was evaluated by assessing intra-run precision and repeatability across serial sections of Tonsil and Colorectal Cancer (CRC) tissue on the BOND RX autostainer by Leica Biosystems. For assessment of intra-run precision (Figure 3), 7 serial sections were stained within the same Bond RX run, imaged and analyzed in order to calculate the coefficient of variation (CV) for intra-run densities (number of positive cells/$mm^2$) and intra-run mean positive cell intensities. Inter-day precision (Figure 4) was assessed by staining 3 serial sections across 3 different days on multiple Bond RX staining runs. Stained slides were imaged and analyzed to calculate the CV for the same two key metrics: cell densities and mean positive cell intensities. Staining performance was found to be qualitatively and quantitatively concordant across all slides within a single run (intra-run precision testing) as demonstrated by CVs of both positive cell density and signal intensity. CVs for positive cell density and mean positive cell intensity were within the acceptance value of 20% for both the positive control tonsil (4.2% and 7.0%, respectively) and the CRC tissue (15.2% and 11.3%, respectively). Similarly, staining performance demonstrated high concordance between slides stained across multiple runs. CVs for positive cell density and mean positive cell intensity remained within 20% for both tonsil tissue (2.5% and 6.7%, respectively) and CRC tissue (13.1% and 11.5%, respectively) across the three independent runs.

We demonstrated excellent assay performance for both the positive control tissue and tumor tissue for which CVs were much lower than the highest accepted value of 20%[5]. To note, for tumor indications, the acceptance range is usually increased to 30% to accommodate possible tumor heterogeneity.

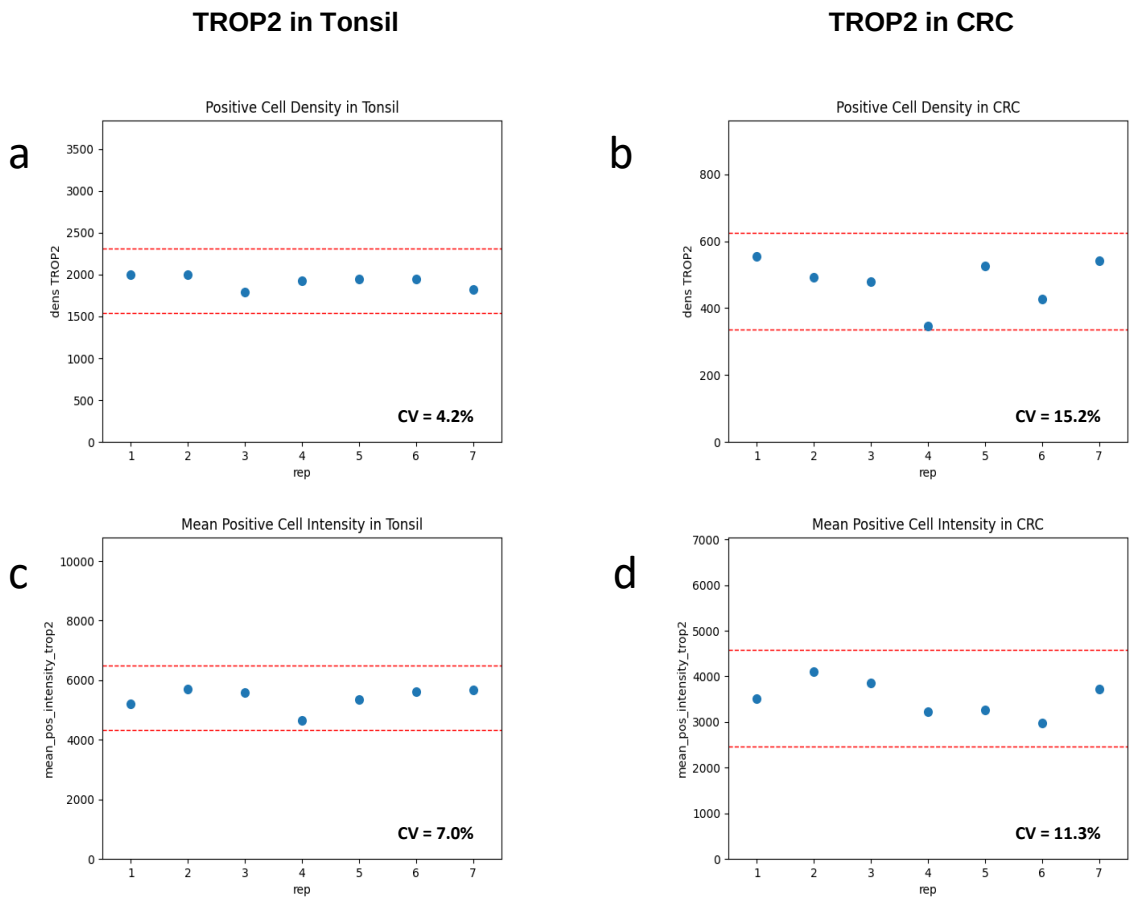



**Figure 3: Intra-run precision of the InSituPlex® monoplex assay a.** Number of positive cells/mm$^2$ per slide on tonsil tissue. **b.** Number of positive cells/mm$^2$ per slide on CRC tissue. **c.** Mean positive signal intensity per slide on tonsil tissue. **d.** Mean positive signal intensity per slide on CRC tissue. For tonsil tissue, thresholds (red dotted lines) mark +20% and -20% of the mean of all samples. For CRC tissue, thresholds (red dotted lines) mark +30% and -30% of the mean of all samples.

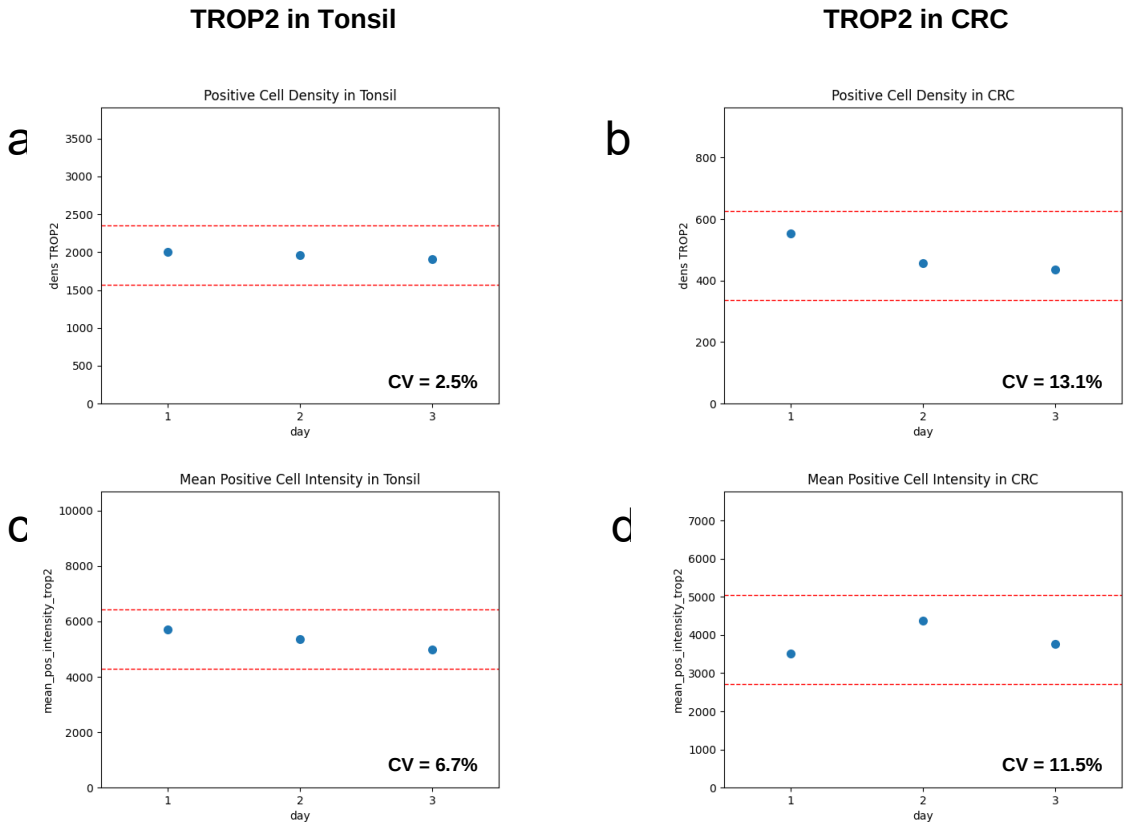



**Figure 4: Inter-run precision of the InSituPlex® monoplex assay a.** Number of positive cells/mm$^2$ per slide on tonsil tissue. **b.** Number of positive cells/mm$^2$ per slide on CRC tissue. **c.** Mean positive signal intensity per slide on tonsil tissue. **d.** Mean positive signal intensity per slide on CRC tissue. For tonsil tissue, thresholds (red dotted lines) mark +20% and -20% of the mean of all samples. For CRC tissue, thresholds (red dotted lines) mark +30% and -30% of the mean of all samples.

## References


1. P. Zeng, M.B. Chen, L.N. Zhou, M. Tang, C.Y. Liu, P.H. Lu, Impact of TROP2 expression on prognosis in solid tumors: a systematic review and Meta-analysis, Sci. Rep. 6 (2016) 33658, https://doi.org/10.1038/srep33658.
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3. Liu, S., Hawley, S.J., Kunder, C.A. *et al.* High expression of Trop2 is associated with aggressive localized prostate cancer and is a candidate urinary biomarker. *Sci Rep* 14, 486 (2024). https://doi.org/10.1038/s41598-023-50215-z
4. Sebastian Foersch, et al (2024) TROP2 in colorectal carcinoma: associations with histopathology, molecular phenotype, and patient prognosis. *The Journal of Pathology: Clinical Research* https://doi.org/10.1002/2056-4538.12394
5. Characterizing Intra-Tumor and Inter-Tumor Variability of Immune Cell Infiltrates in Murine Syngeneic Tumors Mojtahedzadeh, Sepideh et al. The American Journal of Pathology, Volume 191, Issue 12, 2133 - 2146