Biomarker Datasheet

Human CTLA-4 OmniVUE™ Biomarker

Product Overview

CTLA-4 (Cytotoxic T-Lymphocyte Associated Protein 4) is an immune checkpoint receptor expressed on T cells. It plays a crucial role in downregulating immune responses. By binding to its ligands B7-1 (CD80) and B7-2 (CD86) on antigen-presenting cells, CTLA-4 outcompetes CD28, thereby delivering inhibitory signals to T cells. This mechanism is vital for maintaining immune homeostasis and preventing autoimmunity. Recent research has highlighted the importance of CTLA-4 in cancer immunotherapy, where its inhibition can enhance anti-tumor immune responses.

Overview

Target	Other names	Isotype	Primary cell type	Subcellular location	Positive Control(s)
CTLA-4	CD152, CELIAC3, CTLA-4, GSE, IDDM12	Rabbit IgG	T cells, regulatory T cells	Plasma Membrane	Tonsil/Spleen, Lymph Node

*Clone available upon request

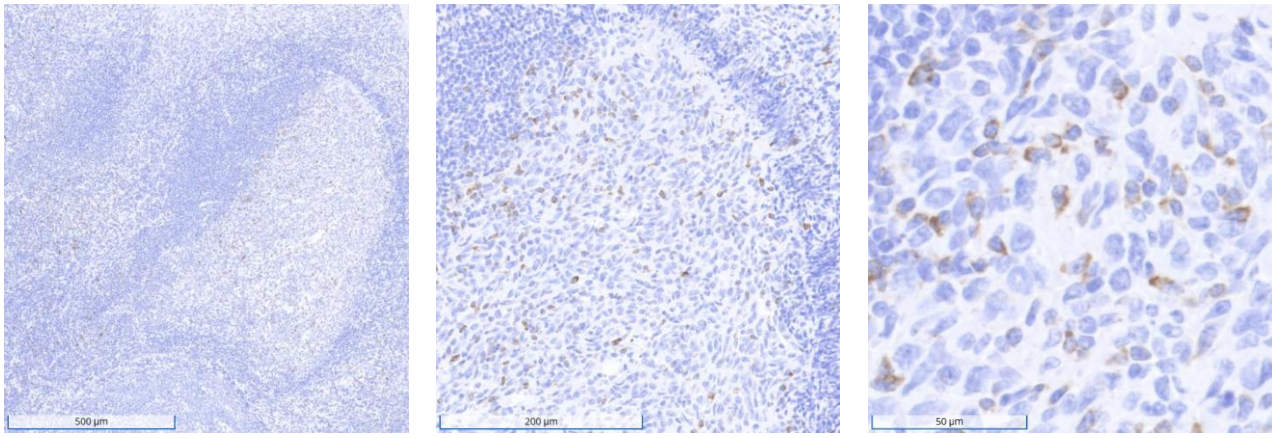
Quality Control

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 tonsil and tumor tissue controls were stained with traditional chromogenic DAB using unconjugated antibodies and with the InSituPlex® (ISP) monoplex assay to demonstrate concordance between staining modalities.

CTLA-4 DAB staining in Tonsil



CTLA-4 ISP staining in Tonsil

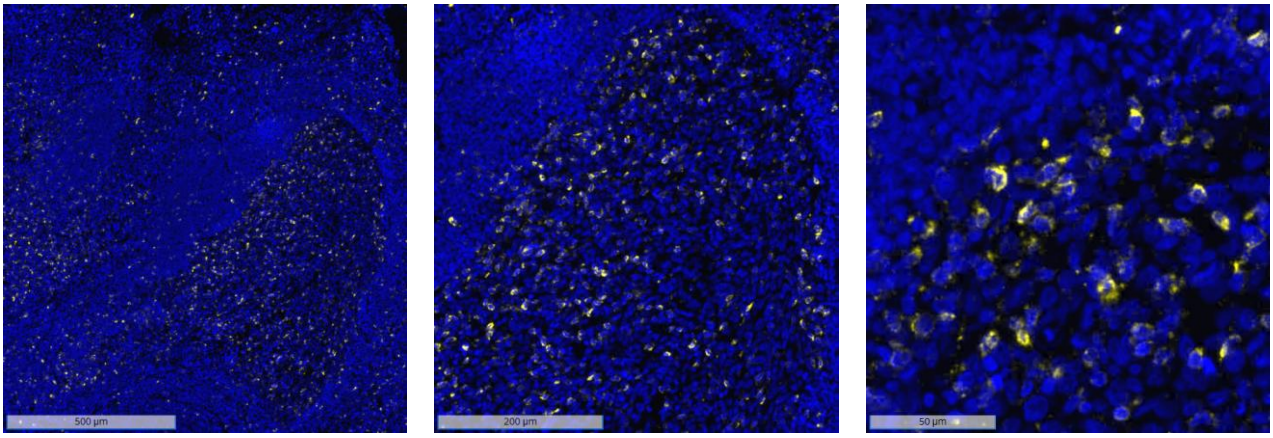
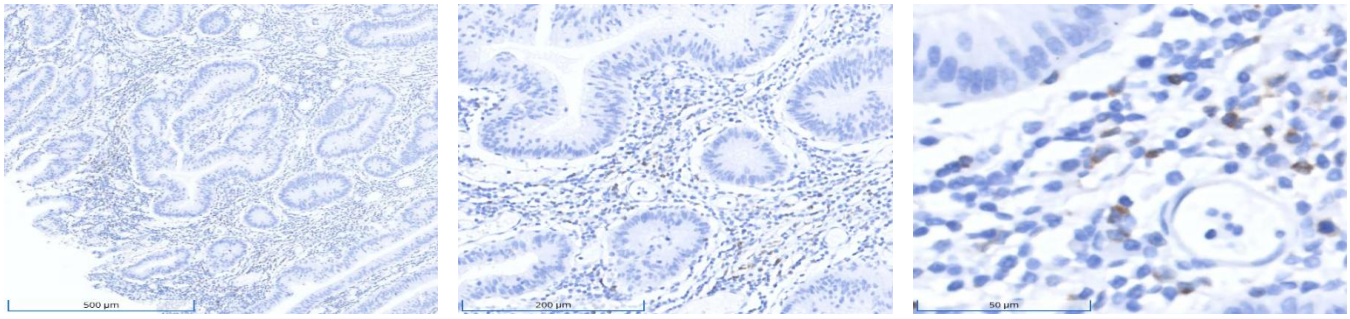


Figure 1: Comparison of unconjugated DAB and InSituPlex® monoplex assay in Tonsil tissue. Chromogenic DAB (top panel), fluorescent ISP staining (bottom panel).

CTLA-4 DAB staining in Colorectal Cancer (CRC)



CTLA-4 ISP staining in Colorectal Cancer (CRC)

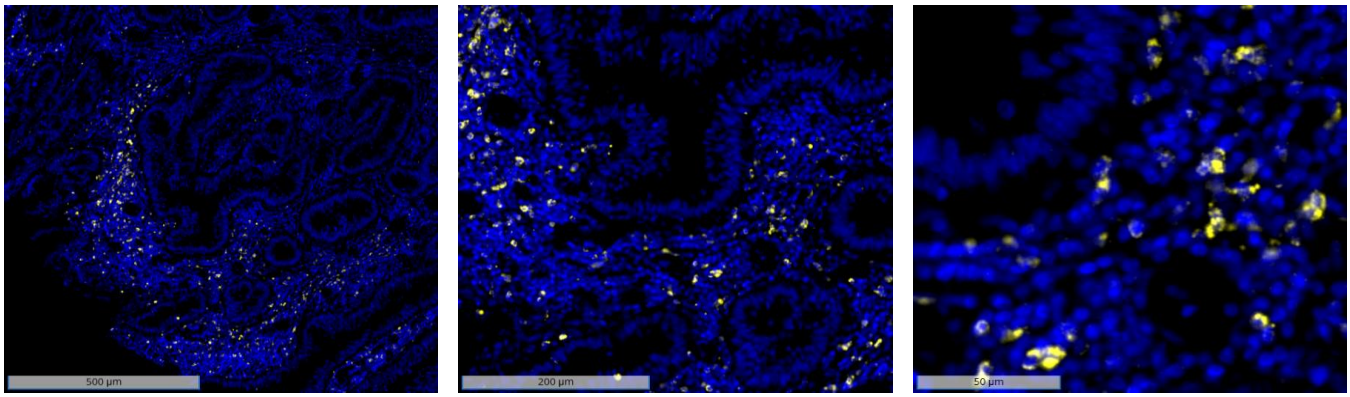


Figure 2: Comparison of unconjugated DAB and InSituPlex® monoplex assay in CRC tissue. Chromogenic DAB (top panel), fluorescent ISP staining (bottom panel).

Assay Performance testing

Precision of the InSituPlex® monoplex assay was evaluated by assessing intra-run precision and repeatability across serial sections of tonsil and 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 the positive control tonsil (5.1% and 7.0%, respectively) and the acceptance value of 30% for the CRC tissue (8.3% and 6.7%, respectively) (Figure 3). 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 tonsil tissue (12.9% and 7.4%, respectively) and within 30% for CRC tissue (6.7% and 10.9%, respectively) across the three independent runs (Figure 4).

We demonstrated excellent assay performance for both the positive control tissue and tumor tissue for which CVs were below the highest accepted value of 20% CV[6] for normal tissue and 30% CV for tumor indications. To note, for tumor indications, the acceptance range is increased to 30% to accommodate possible tumor heterogeneity.

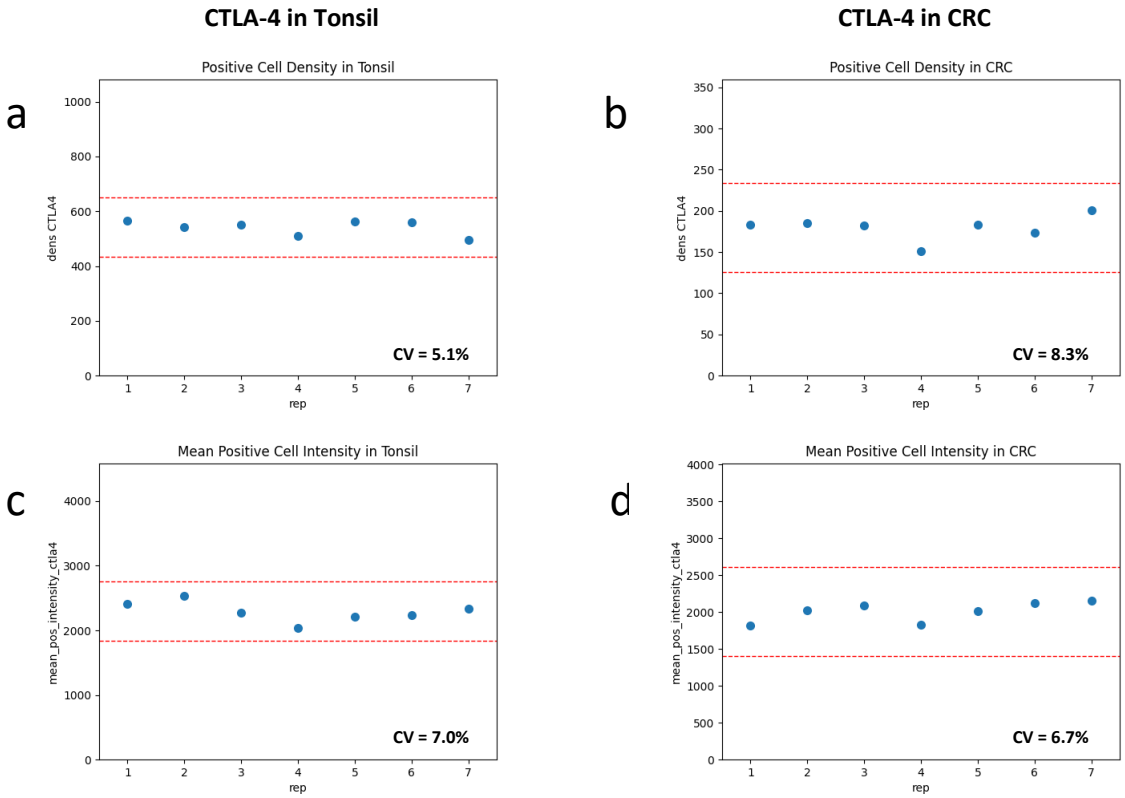


Figure 3: Intra-run precision of the InSituPlex® monoplex assay a. Number of positive cells/mm^2 per slide on tonsil tissue. **b.** Number of positive cells/mm^2 per slide on CRC tissue. **c.** Mean positive signal intensity per slide on tonsil tissue. **d.** Mean positive signal intensity per slide 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.

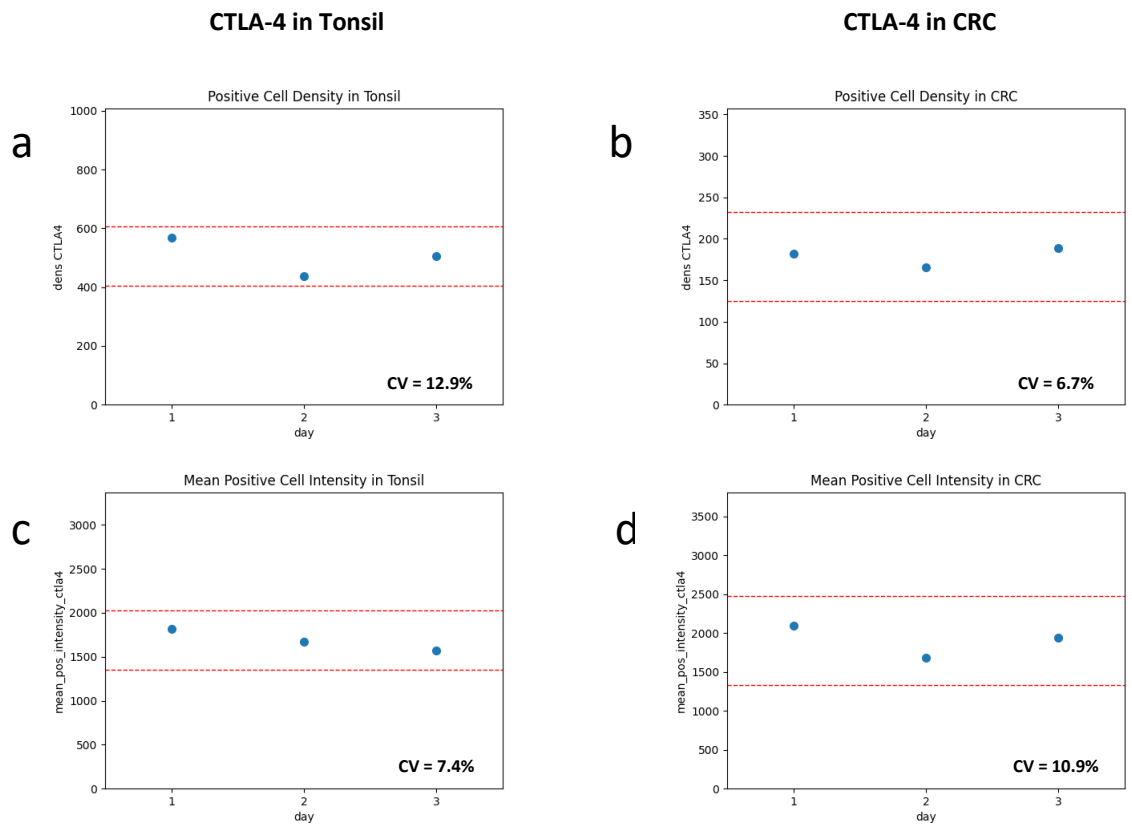


Figure 4: Inter-run precision of the InSituPlex® monoplex assay a. Number of positive cells/mm^2 per slide on tonsil tissue. **b.** Number of positive cells/mm^2 per slide on CRC tissue. **c.** Mean positive signal intensity per slide on tonsil tissue. **d.** Mean positive signal intensity per slide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

References

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