Biomarker Datasheet

Human Lag3 OmniVUE™ Biomarker

Product Overview

LAG3 (Lymphocyte-Activation Gene 3) is an immune checkpoint receptor expressed on activated T cells, regulatory T cells (Tregs), natural killer (NK) cells, and dendritic cells. It functions as a negative regulator of immune responses, similar to CTLA-4 and PD-1. LAG3 binds to MHC class II molecules, inhibiting T cell proliferation and cytokine secretion. Its role in immune regulation makes it an important target in cancer immunotherapy, where blocking LAG3 can enhance anti-tumor immunity. LAG3 has also been implicated in autoimmune diseases and chronic infections.

Overview

Target	Other names	Isotype	Primary cell type	Subcellular location	Positive Controls and relevant indications
Lag3	CD223, Lymphocyte activation gene 3 protein	Rabbit IgG	Activated T cells, regulatory T cells (Tregs), NK cells, dendritic cells	Plasma Membrane	Tonsil Melanoma CRC

*Clone available upon request

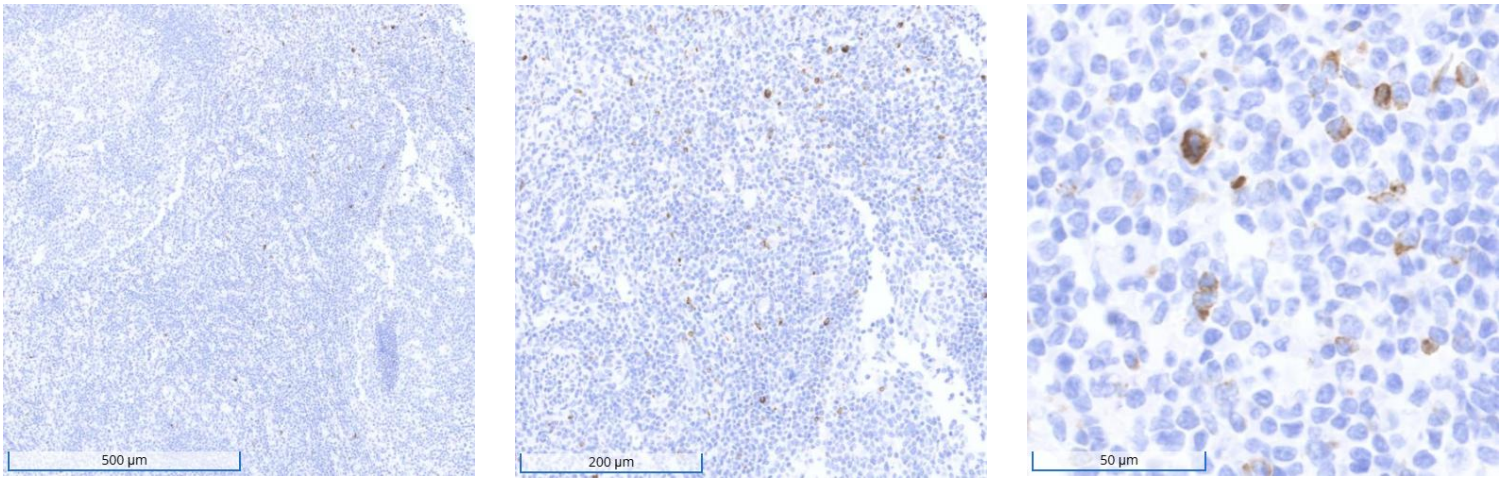
Quality Control

Each lot of antibody conjugate reagent is tested on positive control tissue and reviewed by Ultivue's pathologists and scientists to ensure appropriate staining pattern and signal intensity by both qualitative and quantitative review.

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.

Lag3 DAB staining in tonsil



Lag3 ISP staining in tonsil

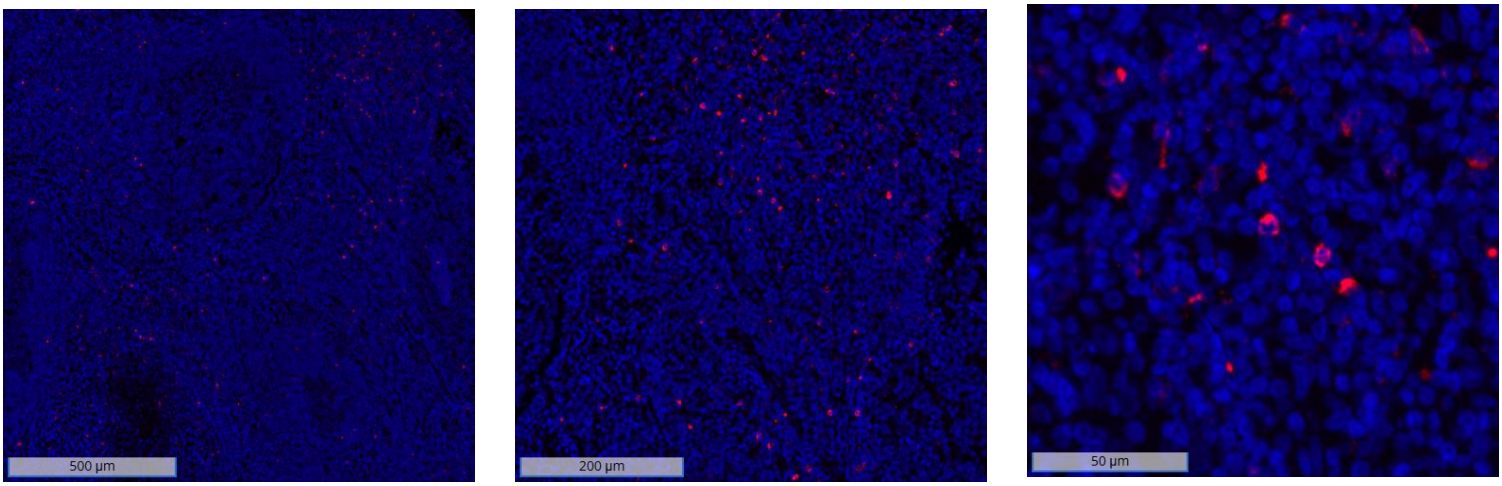
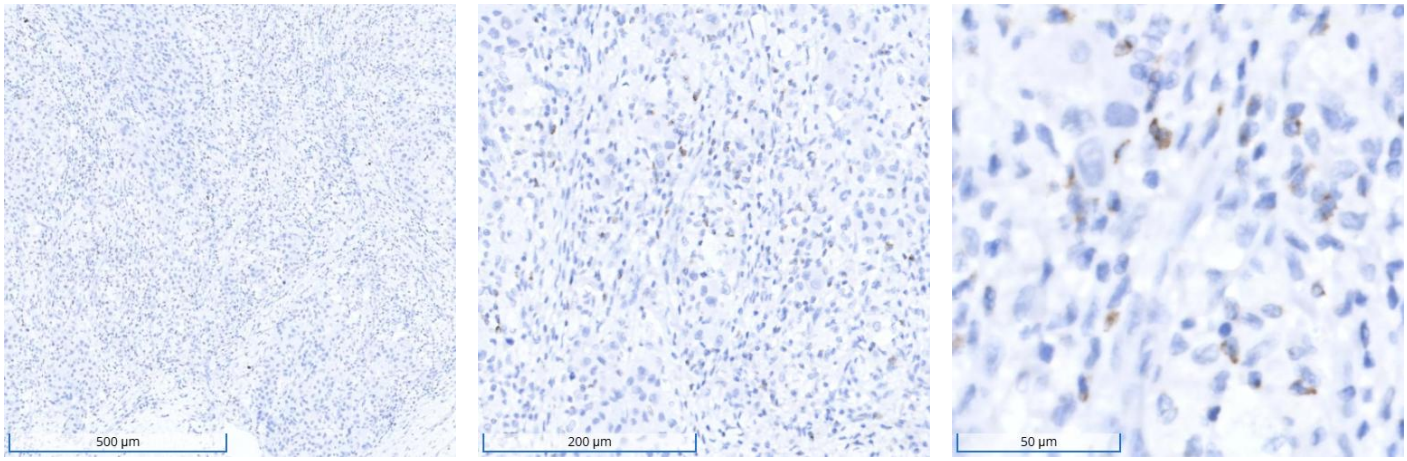


Figure 1: Comparison of unconjugated DAB and InSituPlex® monoplex assay in Tonsil tissue. Chromogenic DAB (top panel), fluorescent ISP staining (bottom panel).

For Research Use Only. Not for use in diagnostic procedures.

Lag3 DAB staining in Melanoma



Lag3 ISP staining in Melanoma

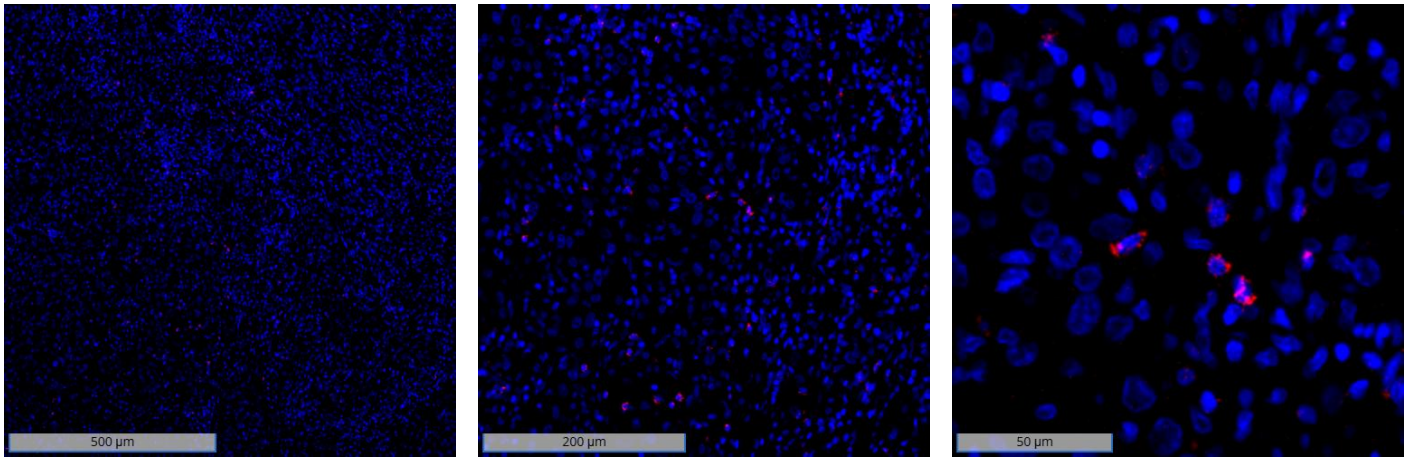


Figure 2: Comparison of unconjugated DAB and InSituPlex® monoplex assay in Melanoma tissue. Chromogenic DAB (top panel), fluorescent ISP staining (bottom panel).

Assay Performance testing

Precision of the InSituPlex® monoplex assay was evaluated by assessing intra-run precision and repeatability across serial sections of tonsil and melanoma 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 (6.4% and 7.7%, respectively) and the acceptance value of 30% for the melanoma tissue (8.3% and 4.3%, 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 (9.1% and 9.4%, respectively) and within 30% for melanoma tissue (19.4% and 10.2%, 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[5] 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.

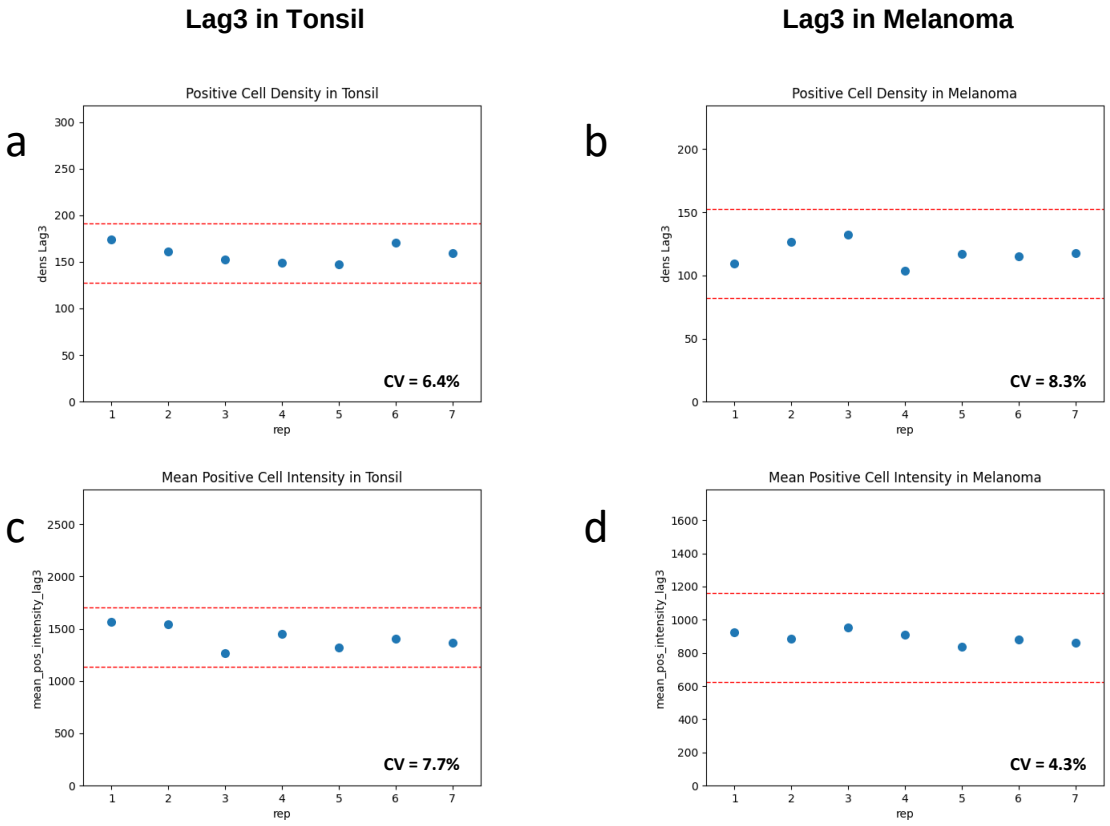


Figure 3: Intra-run precision of the InSituPlex® monoplex assay a. Number of positive cells/mm^2 per slide on tonsil tissue. **b.** Number of positive cells/mm^2 per slide on melanoma tissue. **c.** Mean positive signal intensity per slide on tonsil tissue. **d.** Mean positive signal intensity per slide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

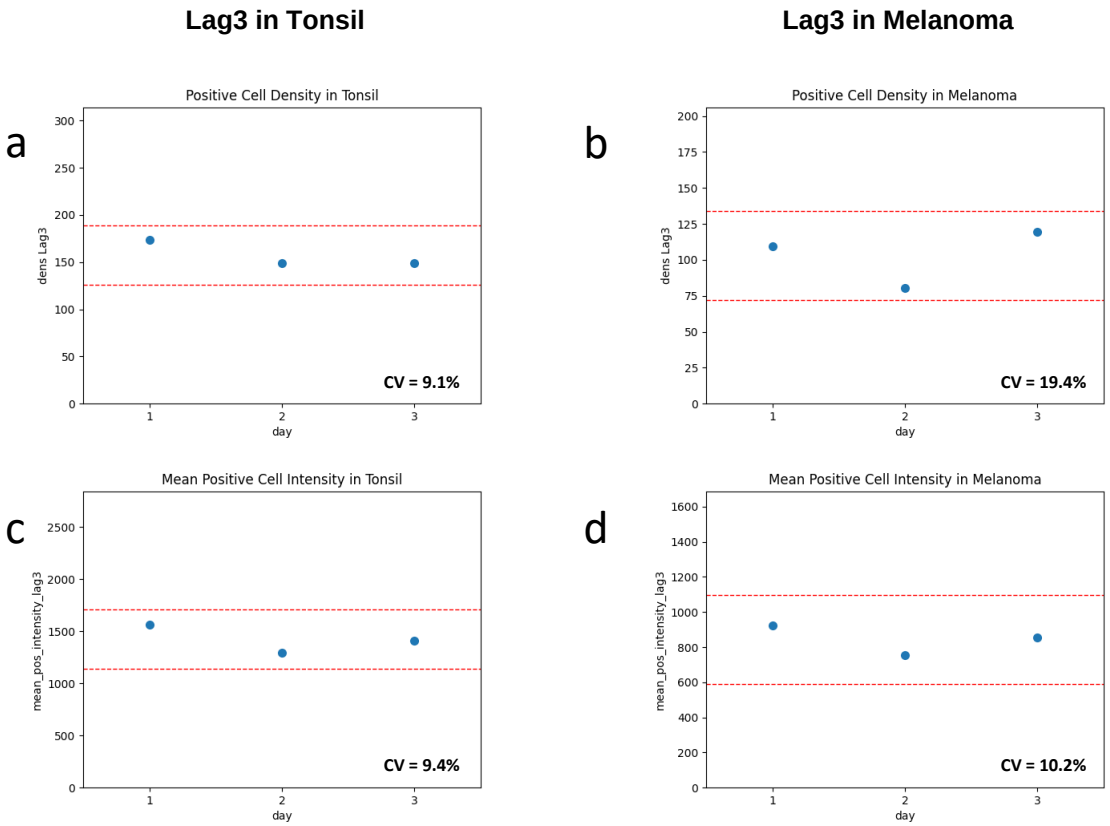


Figure 4: Inter-run precision of the InSituPlex® monoplex assay a. Number of positive cells/mm^2 per slide on tonsil tissue. **b.** Number of positive cells/mm^2 per slide on melanoma tissue. **c.** Mean positive signal intensity per slide on tonsil tissue. **d.** Mean positive signal intensity per slide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

References

1. Workman, C.J., Wang, Y., & Vignali, D.A.A. (2002). LAG-3, a Negative Regulator of T Cell Activation. *Immunological Reviews*, 229(1), 13-25.
2. Grosso, J.F., & Jure-Kunkel, M.N. (2013). LAG3 as a Therapeutic Target in Cancer. *Journal for ImmunoTherapy of Cancer*, 1(1), 16.
3. Woo, S.R., Turnis, M.E., Goldberg, M.V., et al. (2012). Immune Inhibitory Molecules LAG-3 and PD-1 Synergistically Regulate T-cell Function to Promote Tumoral Immune Escape. *Cancer Research*, 72(4), 917-927.
4. Andrews, L.P., Marciscano, A.E., Drake, C.G., & Vignali, D.A.A. (2017). LAG3 (CD223) as a Therapeutic Target for Cancer and Autoimmunity. *Nature Reviews Immunology*, 17(2), 63-75.
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