## Ultivue

#### **Biomarker Datasheet**

### Human CD3 U-VUE<sup>®</sup> Biomarker

CD3 multimeric protein composed of 4 subunits ( $\gamma$ ,  $\delta$ ,  $\epsilon$ ,  $\zeta$ ), which are part of the T-cell receptor (TCR). Engagement of CD3 induces downstream signaling events that result in T-cell activation. The specificity of the CD3 antigen for T cells and its appearance at all stages of T cell development makes it an ideal T cell marker for both the detection of normal T cells and T cell neoplasms.

#### Overview

Target	Other names	Isotype	Primary cell type	Subcellular location	Positive control(s)
CD3	T-cell surface antigen T3	Mouse IgG1	T Cell	Plasma membrane	Tonsil/ Spleen

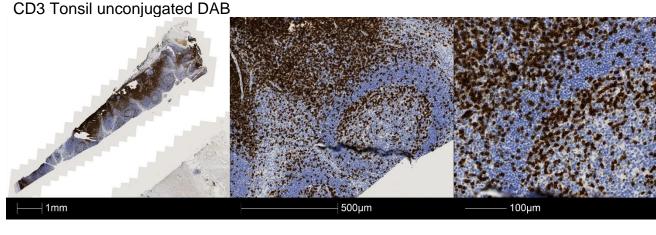
\*Clone available upon request

#### **Quality Control**

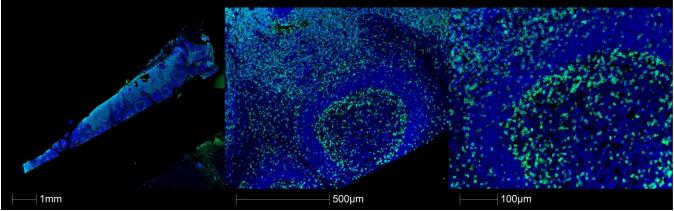
Each lot of antibody conjugate reagent is tested on positive control tissue and reviewed by reviewed by Ultivue's pathologists and scientists to ensure appropriate staining pattern and signal intensity by both qualitative and quantitative review.

#### **Predicate Comparison**

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



CD3 Tonsil ISP

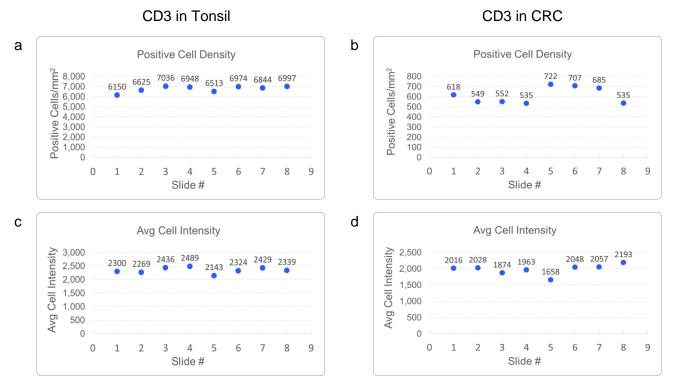


**Figure 1:** Comparison of unconjugated DAB and InSituPlex<sup>®</sup> monoplex assay in tonsil tissue. Chromogenic DAB (top panel), fluorescent ISP staining (bottom panel).

#### Assay Reproducibility

An InSituPlex<sup>®</sup> monoplex assay was performed across serial sections of tonsil and colorectal cancer (CRC) tissue on the Leica BOND RX autostainer. Staining was found to be qualitatively and quantitatively equivalent across all slides in the run as demonstrated by coefficient of variance (CV) of positive cell density and signal intensity.

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**Figure 2: a.** Number of positive cells/mm<sup>2</sup> per slide on tonsil tissue. Inter-slide coefficient of variance (CV) = 4.3%**b.** Number of positive cells/mm<sup>2</sup> per slide on CRC tissue. Inter-slide CV = 12.4% **c.** Mean positive signal intensity per slide on tonsil tissue. Inter-slide CV = 4.4%. **d.** Mean positive signal intensity per slide on CRC tissue. Inter-slide CV = 7.5%.

#### References

- Middelburg, J., Kemper, K., Engelberts, P., Labrijn, A. F., Schuurman, J., & van Hall, T. (2021). Overcoming Challenges for CD3-Bispecific Antibody Therapy in Solid Tumors. Cancers, 13(2), 287. https://doi.org/10.3390/cancers13020287
- Moradi-Kalbolandi, S., Sharifi-K, A., Darvishi, B., Majidzadeh-A, K., Jalili, N., Sadeghi, S., Mosayebzadeh, M., Sanati, H., Salehi, M., & Farahmand, L. (2020). Evaluation the potential of recombinant anti-CD3 nanobody on immunomodulatory function. *Molecular immunology*, *118*, 174–181. https://doi.org/10.1016/j.molimm.2019.12.017
- Yang, Y., Zang, Y., Zheng, C., Li, Z., Gu, X., Zhou, M., Wang, Z., Xiang, J., Chen, Z., & Zhou, Y. (2020). CD3D is associated with immune checkpoints and predicts favorable clinical outcome in colon cancer. *Immunotherapy*, *12*(1), 25–35. https://doi.org/10.2217/imt-2019-0145