**Orion™: 16-Plex Single-Step Stain & Imaging of Oral Squamous Cell Carcinoma**

**Overview**

This is a standard setup for an infiltrating squamous cell carcinoma of the tongue. The procedure involves the staining and imaging of the section. The tissue includes the submucosa, lamina propria, and epithelium. The process involves deparaffinization and antigen retrieval, followed by staining with a panel of antibodies and imaging. The image shows the entire section, allowing for comprehensive analysis of the tissue structure and cellular distribution.

**Normal Tissue**

Scattered T cells (CD3) surround the cancer cells (figure 3). In the normal epithelium, the tumor is highly proliferative, with many Ki67+ cells. Most express E-Cadherin similar to basal mucosal epithelium. Yet in some regions, the tumor cells recapitulate the organizational structure of the normal epithelium. Infiltrating Squamous Cell Carcinoma

**Infiltrating Squamous Cell Carcinoma**

The tumor cells are identified by the expression of E-Cadherin. To identify the boundaries of the tumor, the antibodies are used in the protocol. The tumor stimulates a host fibrocellular immune response that is demonstrated by a mixture of immune cells at the border of the tumor nodule (E-Cadherin in green and Pan-CK in dark blue). Interspersed B cells (CD20, yellow), regulatory T cells, and CD8+ T cells (CD8a, red) are present within the tumor nests. The regulatory T cells are identified by FOXP3 staining of the nucleus. Presentation of a mixture of immune cells is essential for the immune response to be effective.

**T Cell Subsets**

The tumor stimulates the infiltration of T cell subsets, including regulatory T cells (CD4+, FOXP3+), and cytotoxic T cells (CD8+, PD-1+). This is demonstrated by the expression of PD-1 on activated T cells. This region shows an aggregate of CD4+ and CD8+ T cells adjacent to nests of tumor cells marked by E-Cadherin (magenta) and cytokeratin (dark blue) (figure 6). The basal layer of the epithelium can be identified by the expression of E-Cadherin (magenta) and cytokeratin (dark blue). The tumor stimulates a host fibrocellular immune response that is demonstrated by a mixture of immune cells at the border of the tumor nodule (E-Cadherin in green and Pan-CK in dark blue). Interspersed B cells (CD20, yellow), regulatory T cells, and CD8+ T cells (CD8a, red) are present within the tumor nests. The regulatory T cells are identified by FOXP3 staining of the nucleus. Presentation of a mixture of immune cells is essential for the immune response to be effective.

**Submucosal Stroma**

In the submucosal region, there are stromal cells that include endothelial cells (CD31, green), vascular smooth muscle cells (SMA, red), and fibroblasts (FAP, white). The arteriolar blood vessels exhibit a bright orange color due to the presence of SMA and FAP. Vimentin is expressed in many different types of stromal cells.

**Cross-reactivity**

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**Image Analysis**

Orion Squamous Cell Carcinoma Sample Information

- **CD31**: Endothelial cells
- **Pan-CK**: Cytokeratin
- **CD45**: White blood cells
- **CD4**: Helper T cells
- **CD8**: Cytotoxic T cells
- **Ki67**: Proliferative cells
- **E-Cadherin**: Basal cells
- **SMA**: Smooth muscle cells
- **MYH11**: Smooth muscle cells
- **Vimentin**: Fibroblasts
- **FAP**: Fibroblasts

**Image Output**

The output of the imaging process includes high-resolution images that can be analyzed to quantify the presence and distribution of different cell types and molecular markers. This information can be used to understand the biology of the tumor and to develop targeted therapies.