

The HTG EdgeSeq Immuno-Oncology Assay is a next-generation sequencing (NGS) application that measures the expression of 549 genes implicated in the host immune response to tumors. The assay is powered by HTG Molecular Diagnostics' (HTG) proprietary quantitative nuclease protection assay and leverages the high sensitivity and dynamic range of NGS. The HTG EdgeSeq system overcomes a key sample bottleneck for immune response expression analysis using extraction-free, lysis-only chemistry to significantly reduce sample input requirements from limited, precious FFPE tissues, whole blood, and cells. The HTG EdgeSeq instrument automates the nuclease protection step in the library preparation process reducing the number of hands-on steps for fast and easy use of NGS platforms for immuno-oncology expression analysis. The HTG EdgeSeq Immuno-Oncology Assay can be used to measure expression levels of drug targets, tumor infiltrate composition, and total immune cell composition using a single section of FFPE tissue.

549 genes, 10 major groups and pathways



Gain deeper understanding of tumor immune response

- Profile immune response in tumors
- Track expression of drug targets
- Profile FACS cells
- Measure total T-cell load
- Profile T-cell infiltration of tumors
- Analyze tumor microenvironment

Achieve meaningful differential expression analysis of immune markers in melanoma samples

Figure A

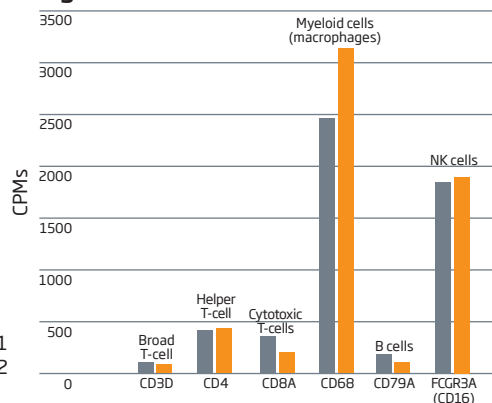
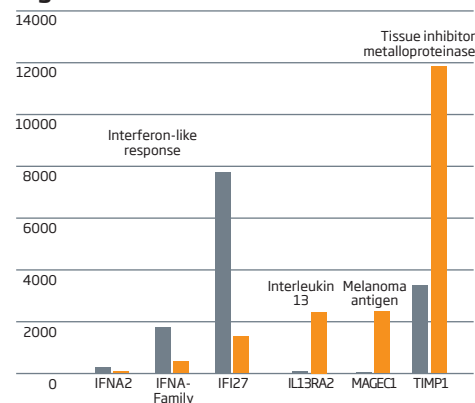


Figure B



Two melanoma FFPE tissue samples were processed with the HTG EdgeSeq Immuno-Oncology Assay using 6.25mm² starting material. Samples were lysed and processed on the HTG EdgeSeq automated system and the resultant NGS libraries were sequenced on the Illumina MiSeq. Data were normalized against all reads per sample and expression level comparison between the two melanoma cases was performed. **Figure A:** Equivalent expression patterns were obtained for CD markers traditionally used for IHC analysis in T, B, Macrophage and NK cells. **Figure B:** When additional markers are examined, two very different immune responses are observed: Lymphocytes in tumor 1 appears to be producing Type 1 interferons, which are generally associated with a positive immune response and slower tumor progression. Tumor 2 on the other hand highly expressed metastasis-related genes generally associated with a poor prognosis.

Benefits

- **Low sample input:** Use just a single 5 µm FFPE tissue section, 32 µL of PAXgene whole blood to obtain comprehensive molecular profile
- **Extraction-free:** Avoid the hands-on time, costs and losses of sample fidelity normally associated with RNA extraction
- **NGS-based:** Leverage the exquisite quantitation capability of NGS platforms to profile 549 key tumor response genes
- **Fewer steps:** HTG EdgeSeq chemistry is fully optimized and requires
 - No** RNA extraction
 - No** cDNA synthesis
 - No** size selection
 - No** rRNA depletion
 - No** end repair
 - No** adapter ligation
- **Rapid results:** From sample to data in less than two days with ~3 hours of hands-on time
- **Simplified data analysis:** From FASTQ to tabular data in 15-30 minutes

Sample input

Sample Type Recommendation

Sample Type	Recommendation
FFPE Tissue	1.56-12.5 mm ²
PAXgene	32 µL
Extracted RNA	10 ng

Ordering information

Illumina (ILM) Next-Generation Sequencing Systems

916-005-208	HTG EdgeSeq Immuno-Oncology Assay ILM (2x8)	2 plates, 8 samples/plate
916-005-008	HTG EdgeSeq Immuno-Oncology Assay ILM (4x8)	4 plates, 8 samples/plate
916-005-224	HTG EdgeSeq Immuno-Oncology Assay ILM (1x24)	1 plates, 24 samples/plate
916-005-024	HTG EdgeSeq Immuno-Oncology Assay ILM (4x24)	4 plates, 24 samples/plate
916-005-096	HTG EdgeSeq Immuno-Oncology Assay ILM (1x96)	1 plates, 96 samples/plate

Ion Torrent (IT) Next-Generation Sequencing Systems

916-005-308	HTG EdgeSeq Immuno-Oncology Assay IT (2x8)	2 plates, 8 samples/plate
916-005-108	HTG EdgeSeq Immuno-Oncology Assay IT (4x8)	4 plates, 8 samples/plate
916-005-324	HTG EdgeSeq Immuno-Oncology Assay IT (1x24)	1 plates, 24 samples/plate
916-005-124	HTG EdgeSeq Immuno-Oncology Assay IT (4x24)	4 plates, 24 samples/plate

