

## QUANTIGENE ASSAY, for multiplex gene expression profiling (GEP)

**Accurate and precise quantitation of  
≤ 80 genes per sample in 96 and 384  
well formats**

### ABOUT QUANTIGENE PLEX ASSAYS

QuantiGene Plex Assays are based on the clinically proven branched DNA (bDNA) signal amplification technology. The assay enables detection and quantitation of multiple RNA targets simultaneously (up to 80 mRNA targets in a single well) using a combination of bDNA signal amplification and Luminex beads (xMAP®) technologies. QuantiGene assays accurately and precisely quantitate RNA, directly from samples without requirements of RNA purification.

### HOW DOES THE ASSAY WORK?

QuantiGene assays utilize branched DNA (bDNA) technology, which has been approved in diagnostic assays for HIV-1 and other diseases.

- Cells or tissue samples are homogenized to release the target RNA.
- Oligonucleotide probe sets are incubated with the target RNA on a capture plate
- The probes cooperatively hybridize to the targets, and capture probes are bound to the beads, capturing the target RNA.
- Signal amplification is performed via sequential hybridization of the bDNA pre-amplifier, amplifier, and label-probe molecules to the target.
- The resulting fluorescence signal associated with individual Capture Beads is read on a Luminex flow cytometer.

### ASSAY BENEFITS

- **Fast sample prep:** perform the assay directly on cell lysates or tissue homogenates. RNA purification is optional
- **Precisely detect subtle changes:** detect gene expression changes smaller than 10%
- **Superior specificity:** delivers greater specificity than other common technologies, and distinguishes between closely related genes due to probe design with several capture points along the target mRNA of interest
- **Flexibility:** amenable to automation for use in routine compound screening

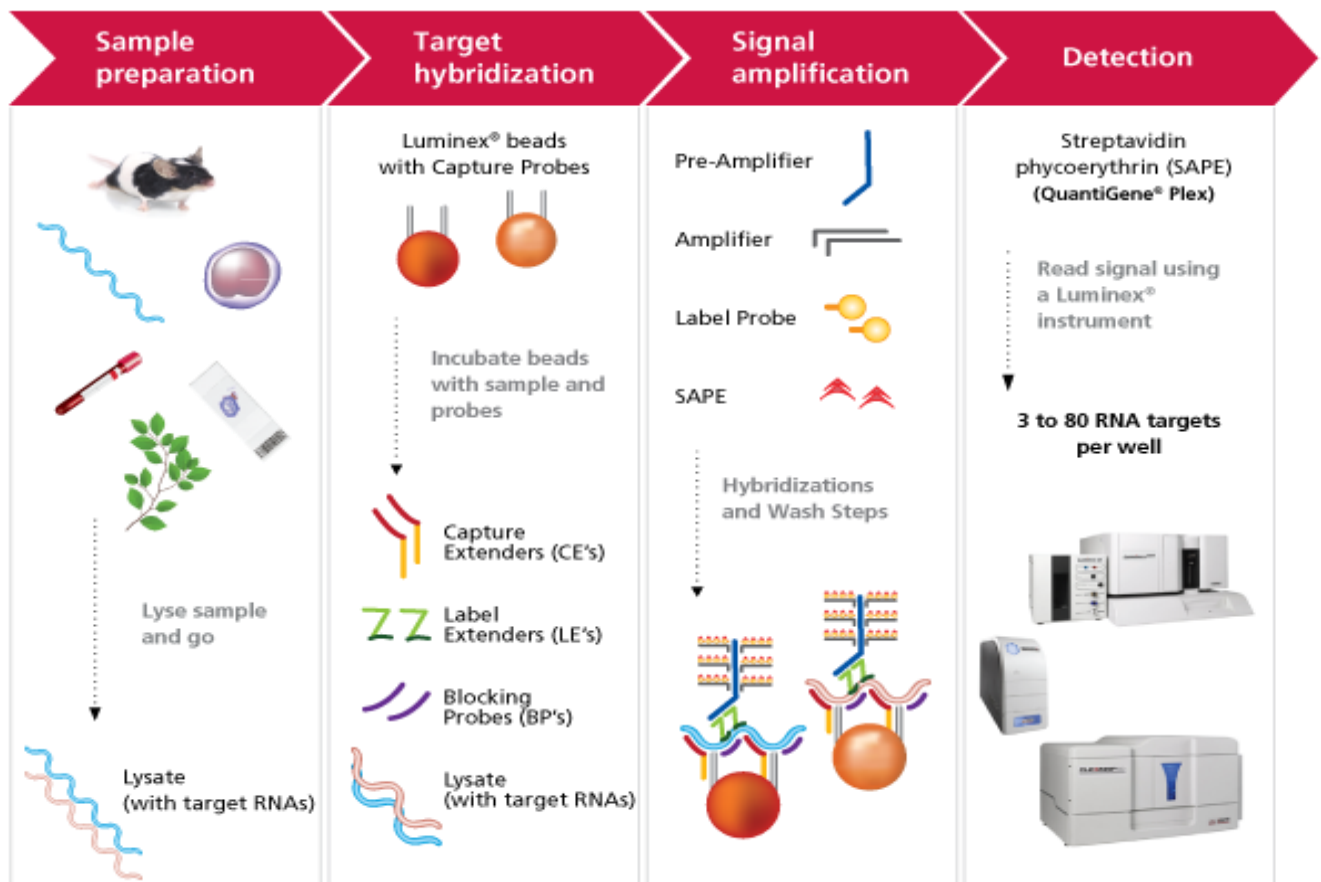


- **True multiplexing:** measure up to 80 genes, including housekeeping genes, in the same well with no cross-reactivity
- **Standardized platform:** 96-well plate format using Luminex assay systems
- **Simple workflow:** ELISA-like workflow for direct hybridization of transcripts to beads and transcript labeling
- **Housekeeping genes (HKG):** multiple HKGs of different abundancy can be used as normalization controls for more reliable data analysis

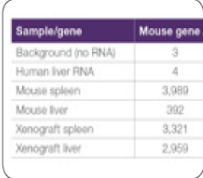
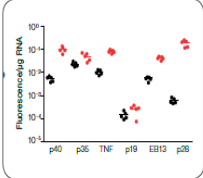
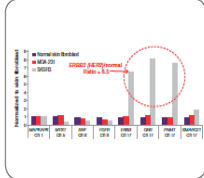
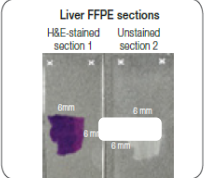
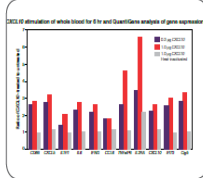
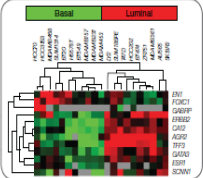
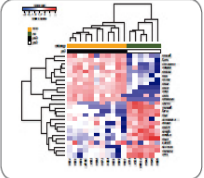
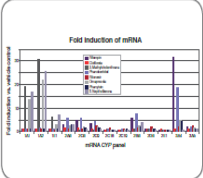
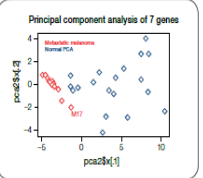
### SAMPLE REQUIREMENTS

- **Minuscule samples:** few cells or small piece of tissues
- **Compatibility:** works with a variety of sample types, such as cultured cells, whole blood, dried blood spots, fresh or frozen animal or plant tissues, purified RNA and heavily degraded and cross-linked RNA in formalin-fixed, paraffin-embedded (FFPE) tissues, etc.

### FOUR MAIN STEPS OF QUANTIGENE ASSAYS



## VARIOUS APPLICATIONS OF QUANTIGENE ASSAY

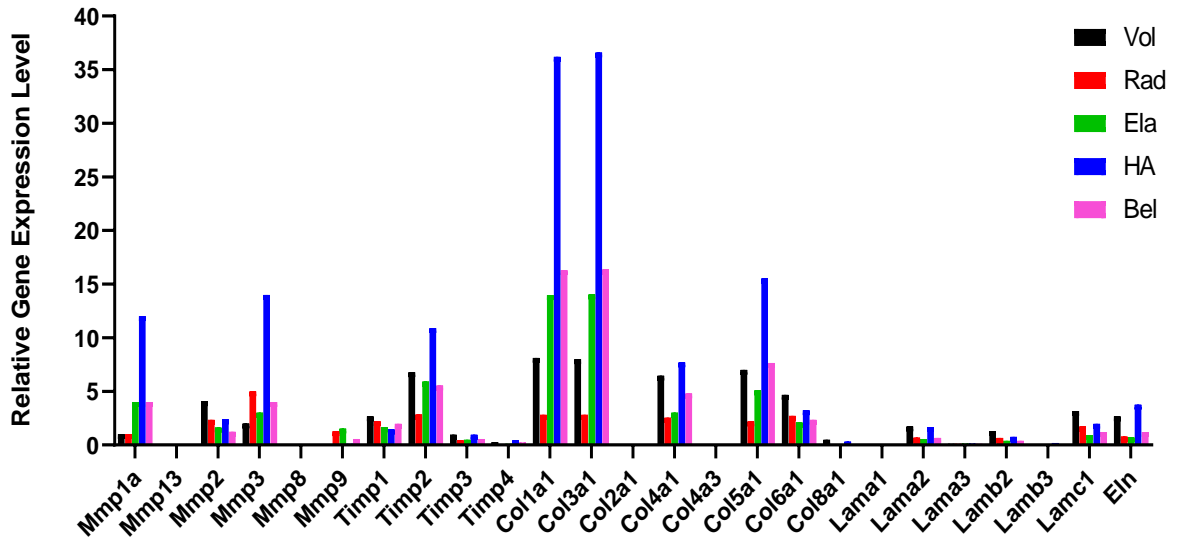
Target discovery Target identification and verification of cell-based microarrays	Lead optimization High-throughput screening Secondary screening	Preclinical studies ADME Toxicology	Clinical studies Biomarker verification Clinical trials (FFPE tissues)															
 <table border="1"> <thead> <tr> <th>Sample/gene</th> <th>Mouse gene</th> </tr> </thead> <tbody> <tr> <td>Background (no RNA)</td> <td>3</td> </tr> <tr> <td>Human liver RNA</td> <td>4</td> </tr> <tr> <td>Mouse spleen</td> <td>3,980</td> </tr> <tr> <td>Mouse liver</td> <td>392</td> </tr> <tr> <td>Xenograft spleen</td> <td>3,321</td> </tr> <tr> <td>Xenograft liver</td> <td>2,959</td> </tr> </tbody> </table>	Sample/gene	Mouse gene	Background (no RNA)	3	Human liver RNA	4	Mouse spleen	3,980	Mouse liver	392	Xenograft spleen	3,321	Xenograft liver	2,959				
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Patient-derived xenograft (PDX) models	Verification of genes involved with inflammation	DNA copy number for breakpoint analysis	Verification of genes in FFPE samples	Expression analysis from whole human blood														
																		
Tumor cell line characterization for drug compound screening	FFPE analysis of genes involved with cancer	CYP genes used with human hepatocytes	PCA analysis of genes involved with melanoma															

### SERVICE FEATURES

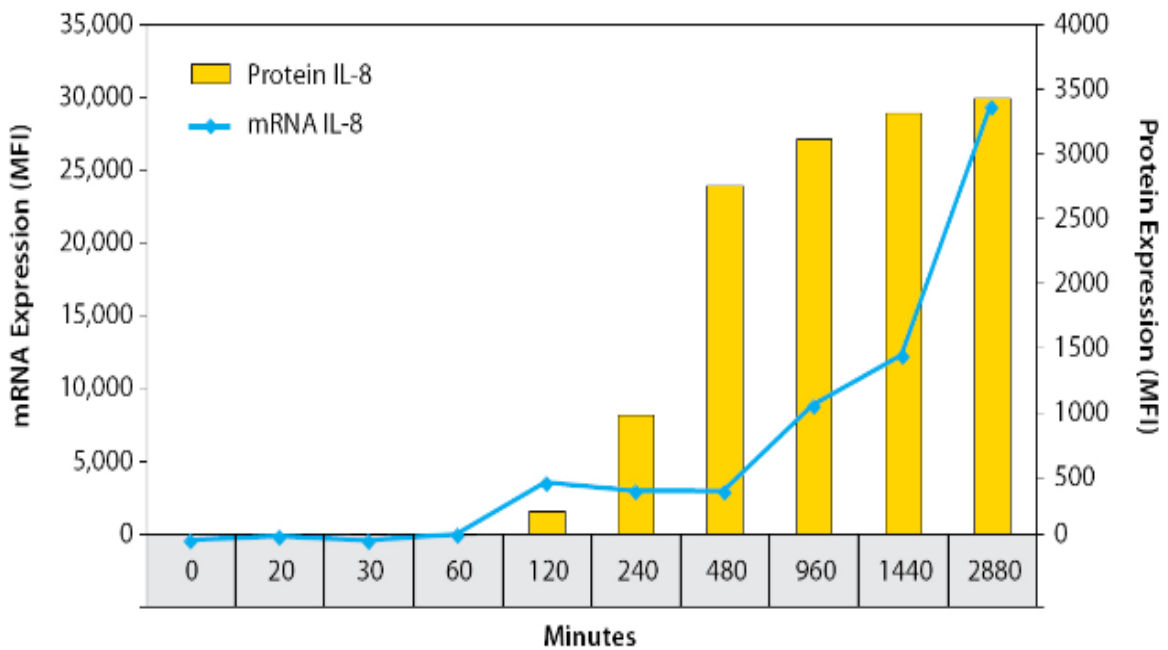
- ❖ **High throughput:** 96 and 384 -well formats
- ❖ **Simultaneous quantitation:** assay both mRNA and protein levels in cells or cell culture supernatants
- ❖ **Highly reproducible**
- ❖ **Flexible:** we can quantify any gene/ protein of interest. Targets of interest and their layout are custom designed so we can deliver data from as few or as many samples / replicates as needed
- ❖ **State-of-art platforms:** Luminex-200, FlexMAP 3D; VorTemp™ 56 Shaking Incubator or Hybridization Oven
- ❖ **Large inventory of verified genes:** over 20,000 genes can be mixed to create pathway- and disease-themed panels, custom probes designed to any sequence within a few days
- ❖ **Extensive data analysis**
- ❖ **Timely data delivery:** 1-4 weeks or sooner- dependent upon receiving test samples
- ❖ **30+ years of accumulated experience:** Expert data analysis and interpretation, high quality scientific and technical support
- ❖ **Optional tests**
  - ✓ We offer qPCR for validation of any gene of interest
  - ✓ RNA samples can be checked for integrity and quality (IQ assay) before gene array analysis
  - ✓ We have a library of >4000 validated **antibodies** to examine samples at protein levels using quantitative Automatic WB.
  - ✓ We offer ~100 **human cancer cell lines and many types of primary cells** for testing drugs and biologicals for specific projects



### ECM Gene Expression Levels from Rat Skin Homogenates



**Example 1. Extracellular matrix (ECM) gene expression levels in rat skin samples** following various treatments (n=12). Results are average fold change of gene expression levels; treated samples (5 indicated treatments) were analyzed relative to untreated samples.



**Example 2. IL-8 expression in human lymphoma cells.** U-937 cells were stimulated for 0 to 48 h. Cells culture supernatants were collected and the corresponding cells were lysed. The culture supernatants and lysates were analyzed for various cytokines using Luminex-based multiplex cytokine assay and QuantiGene assay. Representative data (IL-8) is shown in the graph.

