

QuantiGene®Plex 2.0 Reagent System

Bead based quantitative gene expression analysis: simultaneous and quantitative detection of 3-30 target-specific RNA molecules in different sample types

The QuantiGene Plex 2.0 assays combine branched DNA (bDNA) signal amplification technology and xMAP® (multi-analyte profiling) beads to enable the detection and quantitation of multiple mRNA targets simultaneously. The bDNA assay is a hybridization-based method of target-specific RNA quantitation that amplifies signal rather than target RNA, using labeled DNA probes.

Assay principle

The QuantiGene Plex 2.0 system utilizes fluorescent microspheres (Capture Beads) as a support to capture specific RNA molecules. The ability to quantify multiple target-specific RNA molecules in a single sample lies in the design of the Probe Sets. For each RNA molecule of interest, an oligonucleotide Probe Set containing three types of synthetic probes, Capture Extenders (CEs), Label Extenders (LEs), and Blockers (BLs) that hybridize and span contiguous sequences of the target RNA, is provided. The CEs discriminate among the different Capture Beads within the bead array while capturing, via cooperative hybridization, the target RNA.

Signal amplification is mediated by DNA amplification molecules that hybridize to the tails of the LEs. Each amplification unit contains multiple hybridization sites for biotinylated Label Probes that bind Streptavidin-conjugated R-Phycoerythrin (SAPE). The resulting fluorescence signal associated with individual Capture Beads is read on a Luminex flow cytometer. Signal is reported as mean fluorescence intensity (MFI) and is proportional to the number of target RNA molecules present in the sample.

- Dried Blood Spots
- Whole Blood or PAXgene Blood
- FFPE Sections
- Animal Tissues
- Cultured Cells

The diagram illustrates the assay principle. It shows a Capture Bead (orange sphere) with three types of synthetic probes: Capture Extenders (CEs), Label Extenders (LEs), and Blockers (BLs). mRNA molecules (blue lines) hybridize to the CE and LE regions. In step (a), a 2.0 Pre-Amplifier (green line) hybridizes to the tail of the LE. In step (b), a 2.0 Pre-Amplifier with a biotinylated Label Probe (orange line) hybridizes to the tail of the LE, and Streptavidin-conjugated Phycoerythrin (SAPE) (red sphere) binds to the biotinylated label probe, amplifying the signal.

Step 1: Release Target RNA ➔ Cells are lysed to release RNA.

Step 2: Target RNA Capture ➔ Specific mRNA transcripts are captured to their respective beads through a Capture Extender (CE), addition of Label Extender (LE) for signal maximizing, Capture Probe (CP) interaction during an overnight hybridization at 54°C.

Step 3: Signal Amplification ➔ a) Sequential hybridization of the 2.0 Pre-Amplifier, 2.0 Amplifier and biotinylated Label probe, respectively, for an hour at 50°C
b) Binding with Streptavidin-conjugated Phycoerythrin (SAPE) at room temperature for 30 minutes.

Step 4: Detection ➔ The sample is analyzed on a Luminex® instrument. The level of SAPE fluorescence is proportional to the amount of mRNA transcripts captured by the beads.

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QuantiGene Plex Advantages

- Quantitatively measure multiple RNA targets simultaneously with unparalleled accuracy and precision
- RNA quantitation directly from cultured cells, whole blood, or fresh, frozen or formalin-fixed, paraffin-embedded (FFPE) tissue
- NO RNA purification
- No reverse transcription
- No target amplification
- Simple Assay Workflow

QuantiGene Plex Applications

- Prospective/retrospective studies using whole blood or FFPE samples
- Biomarker validation
- Predictive toxicology
- Microarray validation
- Secondary screening
- RNAi knockdowns and

Create your own target panel using the QuantiGene Plex Set Catalog:
<http://www.panomics.com>

QuantiGene 2.0 Reagent System

The QuantiGene 2.0 Reagent System is composed of several modules, each sold separately, depending on sample type of interest. Each of the modules is sold in a multiple sizes.

Sample Type	QuantiGene 2.0 Assay Kit	QuantiGene Sample	QuantiGene Sample Processing Kit	QuantiGene 2.0 Housekeeping Assessment Kit	QuantiGene 2.0 Probe Sets Gene Panel
Cultured Cells	required	Cultured Cells	n/a	recommended	required
Whole Blood or PAXgene Blood	required	Blood Samples	n/a	highly recommended	required
Fresh/Frozen Animal Tissues	required	Animal Tissues	optional	highly recommended	required
FFPE Tissue Sections or Blocks	required	FFPE Samples	optional	highly recommended	required
Purified RNA	required	n/a	n/a	recommended	required

Ordering Information: QuantiGene Plex 2.0 Assay Kit 96 Tests Cat. No.: Order by request

Test system for simultaneous gene expression analysis: quantitative measurement of 3 – 30 RNA targets in different sample types.

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