Panomics

QuantiGene® for RNA Quantitation

The QuantiGene Reagent System utilizes technology that measures RNA levels directly from cell lysates, eliminating the need to purify RNA or amplify via PCR. QuantiGene sets the highest performance standards yet for RNA quantitation technologies in the areas of precision, accuracy, simplicity and robustness.

- **No RNA Purification**
  Work directly from cell lysates
- **No Reverse Transcription**
  Eliminate biases against messages that do not reverse transcribe well
- **No Target Amplification**
  Eliminate biases against messages that do not amplify proportionately, due to either random events or sequence composition

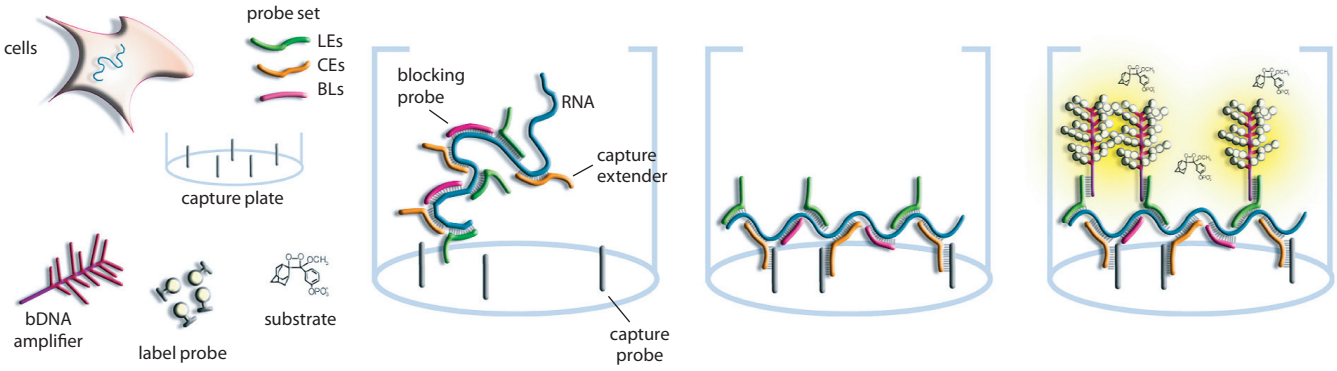
The Technology

QuantiGene Reagent System is a sandwich nucleic acid hybridization assay that provides a unique approach for RNA detection and quantification by amplifying the reporter signal using branched DNA (bDNA) technology. By measuring RNA directly from crude cell lysates or tissue homogenates, the assay avoids variations or errors inherent to extraction and amplification of target sequences. Branched DNA technology is the basis of clinically proven viral load tests commercialized by Bayer Corporation and has been in practice for over a decade in drug discovery and development applications.

Unrivaled Precision

QuantiGene offers unrivaled precision both within and between different experiments and experimental days. Unlike other technologies, QuantiGene will produce the same results even when performed in different labs. Figure 2 demonstrates results of an experiment where U-937 cells were induced with PMA/LPS (Rx) or placebo (no Rx). Each week, cell lysates underwent a freeze-thaw cycle, and mRNA levels were measured for three genes. GAPDH, TNF-α, and IL-8 CVs ranged from 0.3% to 6.4% within an experiment, and 0.5% to 18.9% between experiments conducted over the course of 12 weeks.

Figure 1: Overview of the QuantiGene Reagent System Technology.



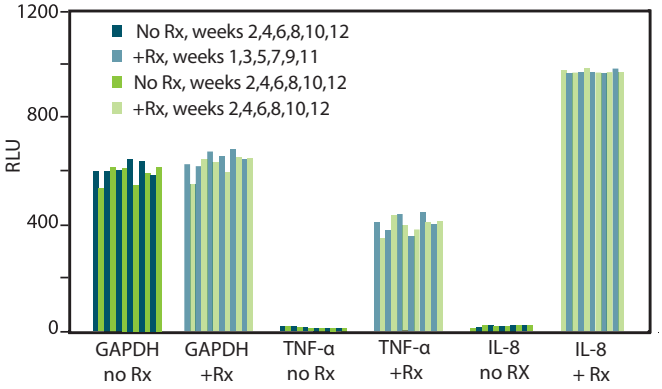
QuantiGene Reagents and starting materials, including cells or tissue

Target mRNA from lysed cells is hybridized to the probe set

Cooperative hybridization of mRNA—probe set mixture to capture plate

Signal amplification with branched DNA, label probe, and substrate

Figure 2: RNA quantitation using frozen cell lysates over a 12-week period.



Treatment Group	Intra % CV	Inter % CV
GAPDH control	2.31	6.05
GAPDH treated	5.79	7.69
TNF-α control	6.39	15.87
TNF-α treated	5.58	7.89
IL-8 control	3.28	15.34
IL-8 treated	0.31	0.35

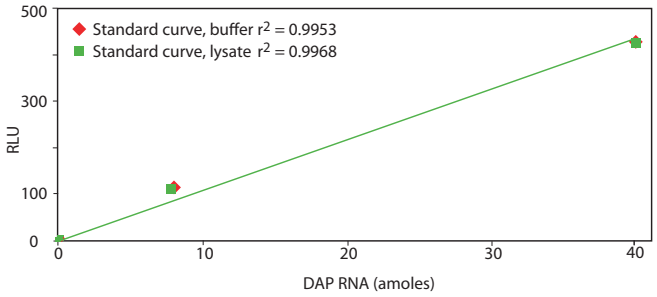
Accuracy

A bacterial control RNA, DAP, was spiked at known concentrations into buffer (red) and HL60 human cell lysates (green). DAP RNA levels were measured and two standard curves plotted (Fig. 3). Recovery of the spiked RNA was 93.8%–99.5% demonstrating the exceptional accuracy of the technology. The standard curves in Fig. 3 span a linear dynamic range of 3.5 logs, and are identical in both buffer and lysate, indicating that the presence of crude cellular lysate does not affect the assay accuracy or precision over a broad range of message levels.

Simplicity

By eliminating the need to purify or amplify RNA, QuantiGene simplifies the process of going from cells or tissues to accurate RNA measurements. Advantages of the technology include reduced handling time, higher recovery of RNA, and the elimination of biases introduced by reverse transcription and PCR amplification. Only general laboratory equipment is required, including a 96-well plate-based luminometer and an incubator capable of maintaining a temperature between 46°C and 53°C.

Figure 3: Spike recovery of DAP RNA in lysate and buffer.



Sample	RLU	Actual (amoles)	Calculated (amoles)	% Recovery
Buffer	22.86	1.6	1.5	93.8
	210.17	20.0	19.2	96.0
Lysate	21.92	1.6	1.5	93.8
	217.07	20.0	19.9	99.5

Ordering Information

The QuantiGene Reagent System is comprised of 2 or 3 modules (each sold separately):

- QuantiGene Assay Kit (Explore, Discover or Screen)
- Target-specific Probe Set(s)
- Sample Processing Kit (not required for most cell lysate preparations)

Contact your local representative about our evaluation program available to new users. QuantiGene Evaluation Kits include: an Explore Kit, two customer specified target-specific probe sets (1 housekeeper and 1 target), and an appropriate Sample Processing Kit. For more information visit our website at www.panomics.com or call us at 1.877.726.6642.

Product	Size	Catalog No.
QuantiGene Evaluation Kit Cultured Cells	2 x 96 assays	QG0005
QuantiGene Evaluation Kit Animal Tissues	2 x 96 assays	QG0006
QuantiGene Evaluation Kit Plant Tissues	2 x 96 assays	QG0007
QuantiGene Evaluation Kit Purified RNA	2 x 96 assays	QG0008
QuantiGene Evaluation Kit Viral Genomic RNA	2 x 96 assays	QG0009

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