DataSheet

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QuantiGene® miRNA Assays

Accurate and precise miRNA quantitation

The importance of miRNA

Since their discovery in 1993, small non-coding RNAs (microRNAs, scaRNAs, and snoRNAs) have emerged as a major component in the regulatory circuitry that underlies the development and physiology of complex organisms. As a result, it is becoming increasingly important to complement messenger RNA (mRNA) gene expression studies with miRNA analysis to understand the biological context of differentially expressed genes.

These key functional gene products are estimated to regulate approximately 30 percent of all protein-coding genes. These small non-coding RNA molecules cover the broadest spectrum of developmental and physiological mechanisms in the cell, including:

- Protein translation inhibition
- Ribosomal RNA processing
- Alternative splicing
- mRNA degradation

The QuantiGene® miRNA Assay provides **direct** measurement with excellent specificity for mature target miRNA. Based on proprietary chemistry for probe sets and branched DNA (bDNA) signal amplification, the assay provides accurate and precise miRNA quantitation.

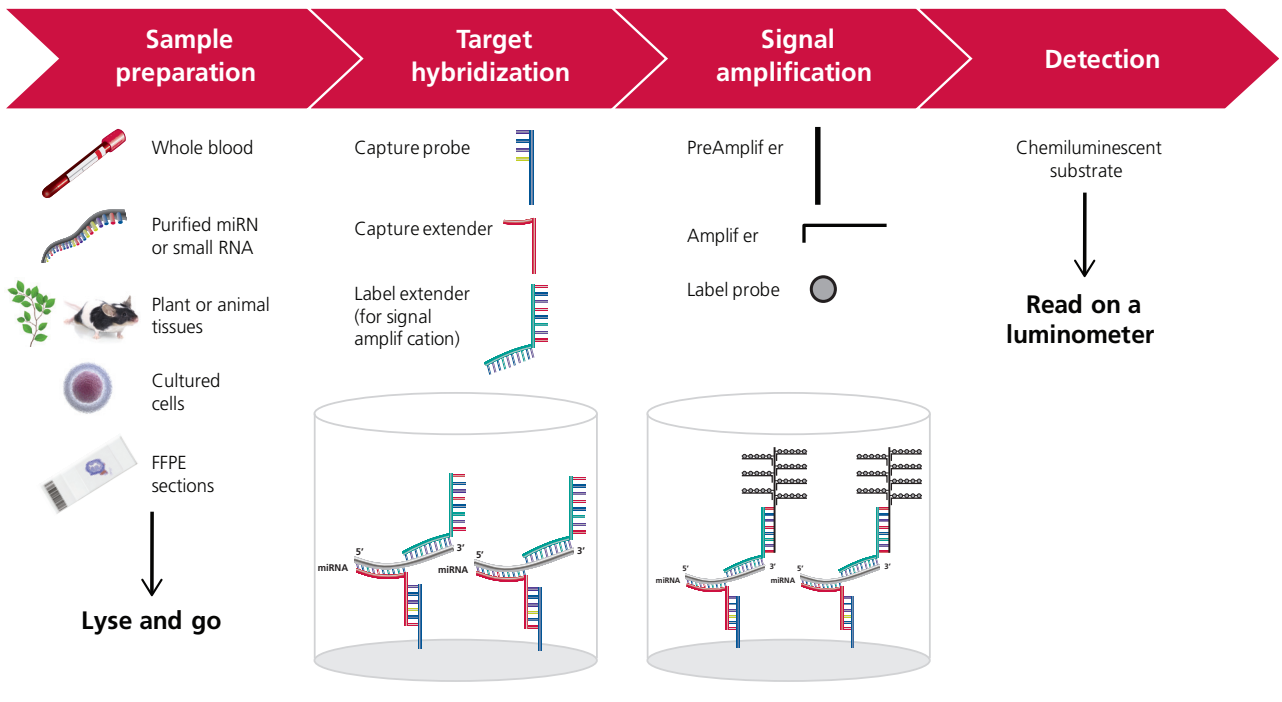
Features and benefits of the QuantiGene® miRNA Assay

- **Validated probe sets**
  - Sensitivity and specificity to targeted mature miRNA and cross-reactivity to closely related family members
- **Avoid biases inherent to purification and amplification**
  - Direct analysis from lysates with no miRNA isolation
  - Based on clinically proven bDNA technology, which relies on signal amplification without cDNA synthesis or PCR amplification
- **Accurate results from a variety of samples**
  - Purified miRNA or small RNA, cultured cells, plant tissues, whole blood, fresh, frozen, and FFPE tissues, and more
- **Probes are designed to the same target sequence as the miRNA probes on the GeneChip® miRNA 2.0 Array**

Assay workflow

The QuantiGene miRNA Assay uses bDNA technology to quantify miRNA directly from the sample without miRNA purification, cDNA synthesis, or PCR amplification. The assay has four main steps: sample preparation, hybridization with probes, signal amplification using bDNA technology, and detection (Figure 1).

Figure 1: The QuantiGene miRNA Assay.



Specifications

Coefficient of variation	<10%
Sensitivity	>3,000 miRNA copies
Specificity	One base
Dynamic range	3 logs
Probe length	25-mer; less if miRNA length is less than 25

Avoid purification biases with direct-from-lysate analysis

To compare QuantiGene® miRNA Assay performance directly between cell lysates and purified RNAs, miRNA miR-31, miR-34a, miR-145, miR-181a, and miR-222 assays were performed on RNA purified from 16,000 HeLa cells using an Ambion mirVana™ miRNA Isolation Kit and cell lysates of the same number of cells. The results were normalized to the direct lysate data for each miRNA assay. The signal of the test miRNAs in cell lysates was 1.8-fold higher than the signal of pure RNAs, indicating that miRNA was lost during the purification step, leading to purification bias in the assay (Figure 2). Similar data were obtained using MCF-7 cells (Figure 3).

Figure 2: Comparison of miRNA levels in purified RNA and in direct cell lysate from 16,000 HeLa cells.

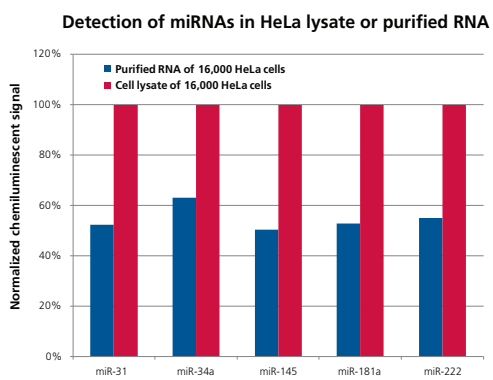


Figure 3: Comparison of miRNA levels in purified RNA and direct cell lysate from 16,000 MCF-7 cells.

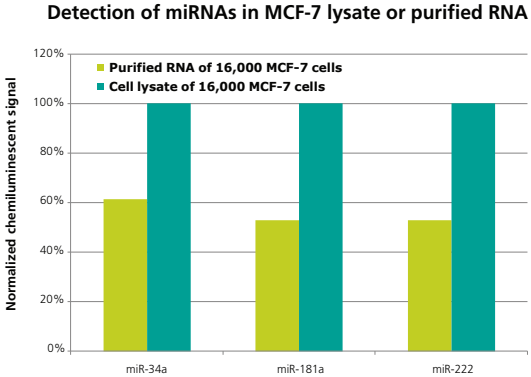


Table 1: Discriminate between closely related family members.

Synthetic target miRNA	Sequence	miRNA probe	Detection
Let-7a	UGAGGUAGUAGGUUGUAUAGUU	Let-7a	100%
Let-7c	UGAGGUAGUAGGUUGUAUGGUU	Let-7a	3%
Let-7f	UGAGGUAGUAGAUUGUAUAGUU	Let-7a	3%
miR-18a	UAAGGUGCAUCUAGUGCAGAUAG	miR-18a	100%
miR-18b	UAAGGUGCAUCUAGUGCAGUUAG	miR-18a	0%
miR-30a	UGUAAACAUCCUCGACUGGAAG	miR-30a	100%
miR-30b	UGUAAACAUCCCCGACUGGAAG	miR-30a	1%
miR-30c	UGUAAACAUCCUUGACUGGAAG	miR-30a	1%

High specificity to target miRNA

The ability of the QuantiGene miRNA Assay to distinguish highly homologous targets with a single-base difference was evaluated with synthetic miRNAs of the hsa-let-7 family, miR-18a/b, and the miR-30 family (Table 1) and other miRNAs (data not shown). Very low levels of non-specific signal were observed for many miRNAs differing by one or two bases. For analysis, assay signals were normalized to the perfect match combination of probe and target for each assay.

High specificity for mature miRNA

The QuantiGene miRNA Assay can differentiate mature miRNAs from longer precursor pre-miRNAs. miRNA probe sets were designed and assayed with mature and pre-miRNA target molecules. The miRNA probe sets only showed less than 5 percent signal with pre-miRNA targets when analyzing samples containing 600,000 copies. These results indicated that the miRNA probe design is highly specific for mature miRNA (Figure 4).

Figure 4: Discrimination between mature and precursor miRNAs.

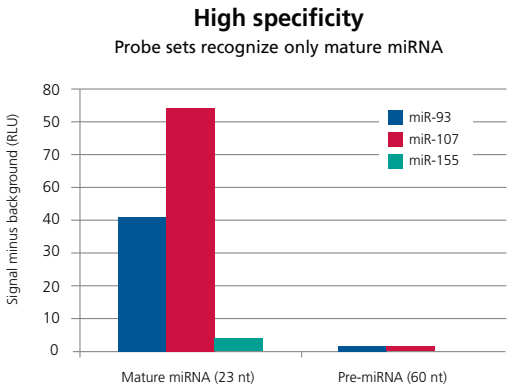
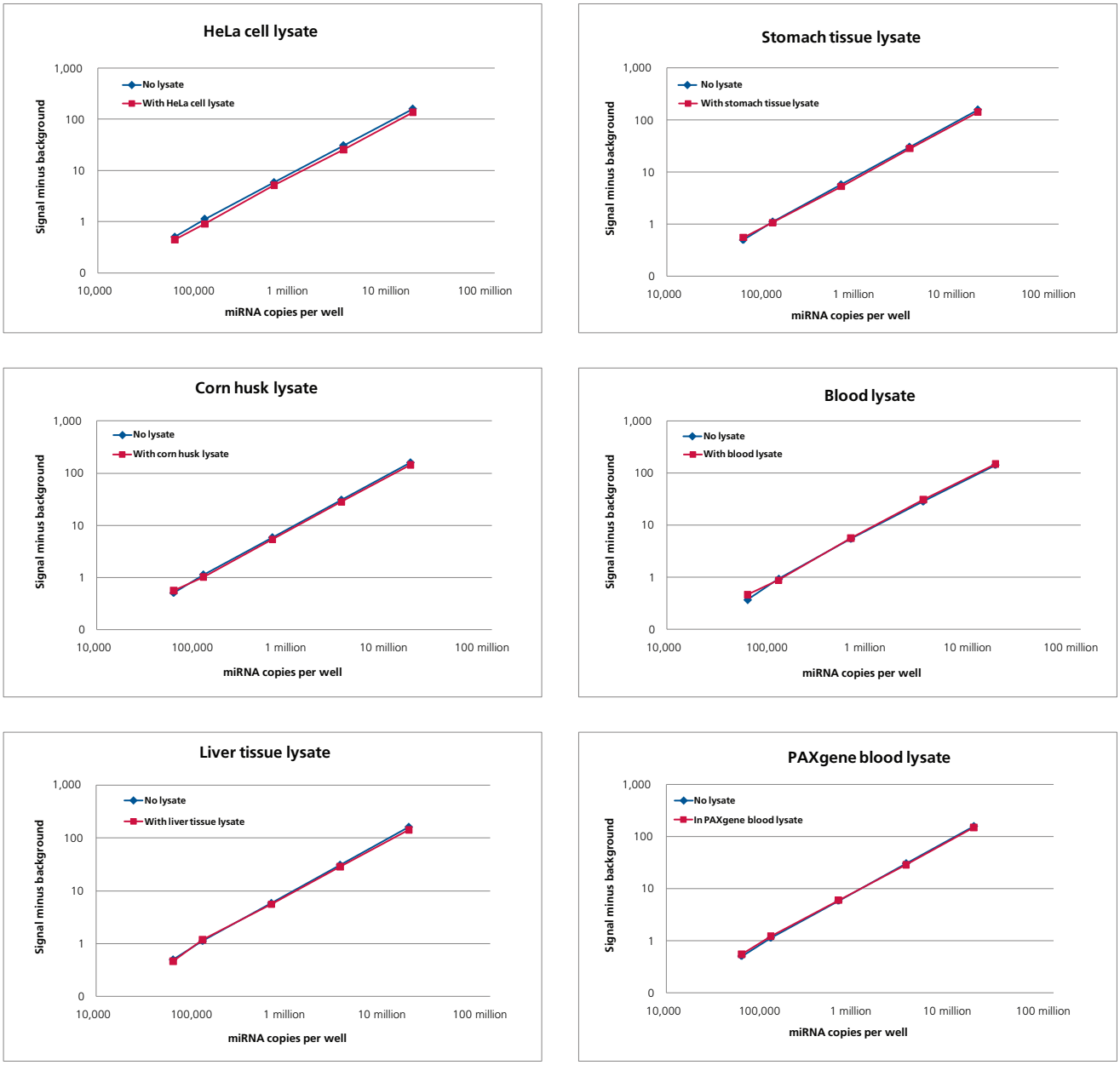


Figure 5: Assay linearity and spike recovery data. Data from spike recovery of a 23 bp unique synthetic miRNA sequence in different sample types.



Accurate quantitation in a variety of samples

The QuantiGene® miRNA Assay offers excellent spike recovery for miRNA with a ±20 percent recovery rate. The spike recovery rates are excellent across a variety of sample types: cell lysates, corn husk lysate, liver tissue lysate, stomach tissue lysate, blood lysate, and PAXgene blood lysate (Figure 5). The assay linearity and spike recovery were tested using a 23 bp unique synthetic miRNA sequence that is not present in any species.

Concordance with GeneChip® miRNA 2.0 Array The QuantiGene miRNA Assay can be used to validate GeneChip miRNA 2.0 Array results. To evaluate the concordance of fold changes between QuantiGene miRNA Assays and GeneChip miRNA 2.0 Array data, a regression analysis of fold differences was performed using Ambion FirstChoice® Human Brain Total RNA (AM7962) and Ambion FirstChoice Human Lung Total RNA (AM7968) using QuantiGene data for 20 miRNAs and GeneChip miRNA 2.0

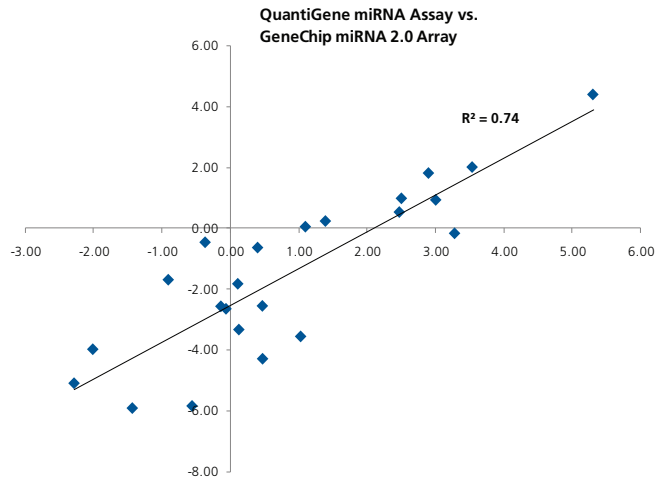
Array data (Figure 6). The correlation coefficient (R^2 value) between these two assays was 0.74, indicating a good fold-change correlation.

Additional information

Each QuantiGene® miRNA Assay is composed of three modules, each sold separately:

- QuantiGene® Assay Kit, which contains all reagents required to perform the QuantiGene® miRNA Assay
- QuantiGene® Sample Processing Kits, which contain components to release and stabilize nucleic acids, available for a variety of sample types (e.g., cultured cells, tissue, blood, and FFPE samples)
- QuantiGene® miRNA Probe Set, which is designed for the miRNA target of choice. The probe sets are functionally validated for sensitivity and specificity.

Figure 6: QuantiGene miRNA Assay and GeneChip® miRNA Array data concordance.



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