A revolution in PCR analysis!

Detect amplicon in seconds
Generate accurate quantitative gene expression data
Eliminate post-PCR processing
Minimize risk of contamination with closed-well detection
Ideal for high-throughput multiplexing

Sentinel™ Molecular Beacons

Fast, convenient qualitative and quantitative PCR analysis
**Sentinel™ Molecular Beacon System for PCR Analysis**

**Applications**
- Determination of target presence/absence
- Quantitative gene expression analysis
- Positive endogenous controls
- Design custom assays
- Allele discrimination
- Single nucleotide mutation detection
- PCR multiplexing

**OUT WITH THE OLD: Gel electrophoresis, semi-quantitative data**
PCR is typically followed by slow, tedious analysis using gel electrophoresis. Mutagenic ethidium bromide has to be handled cautiously. Tubes have to be opened to remove samples, increasing the risk of carry-over contamination. The amount of quantitative data gathered by gel analysis is limited. Now there is a better way to analyze your PCR.

**IN WITH THE NEW: PCR analysis with molecular beacons, truly quantitative data**
PCR analysis with Sentinel™ molecular beacons opens the door to true quantitative analysis while saving time and work by completely eliminating electrophoresis and the need for post-PCR manipulation. Analyzing your PCR has never been so quick or easy.

**Better than gel electrophoresis and other fluorescent PCR systems**
- Faster and easier than electrophoresis
- Truly quantitative PCR analysis
- Minimized risk of carry-over contamination
- Most sensitive PCR system for single nucleotide mismatch detection and allele-discrimination
- Versatile cycling parameters simplify primer/probe design
- High-throughput and multiplexing capabilities
- Higher specificity than systems using fluorescent DNA-binding dyes or primers

**How the System Works:**
Sentinel molecular beacons provide a new method for PCR analysis that is faster and more convenient than ever before:

1. Construct a molecular beacon probe specific for your target.
2. Add the molecular beacon probe to the PCR mixture.
3. Run PCR. As PCR proceeds, product accumulates and the molecular beacon produces fluorescence proportional to the amount of specific amplicon present.
4. Monitor the reaction by measuring fluorescence either as the reaction is occurring or when cycling is complete.

**Qualitative vs. Quantitative Fluorescence Measurements with Molecular Beacons**
Sentinel molecular beacons are well suited for both qualitative (endpoint) and quantitative (real-time) PCR analysis and monitoring. Either may be used for the most basic molecular beacon application, which is determining the presence of a specific amplified product.

**Qualitative PCR analysis** utilizes a fluorometer such as Stratagene’s FluorTracker™ fluorescence reader, which is designed to accommodate all PCR labware (tubes, strips and 96-well PCR plates) and optimized for molecular beacon fluorescence. Qualitative PCR analysis produces accurate results rapidly allowing a single fluorescence reader to easily handle the output of a number of thermal cyclers. When your PCR is completed, you are only minutes from discovering if your amplicon is present. Quantitative assessments based on endpoint fluorescence, however, are inherently inaccurate due to the influences of limiting reagents and small differences in reaction components or cycling parameters.

**Quantitative PCR analysis** requires a spectrofluorometric thermal cycler. Using molecular beacons to monitor PCR reactions in real time provides quantitative data for gene expression analysis and accurate determination of initial copy number. Each cycle produces a fluorescent signal proportional to the amount of amplicon present. Results are typically displayed on an amplification plot showing the change in fluorescence with cycle number. This information may be used to quantify initial target copy number. Studies have shown that initial copy number can be accurately quantified during PCR by determining threshold cycle, that cycle at which the amplification plot crosses a defined fluorescence threshold. Since the threshold cycle is inversely proportional to the log of the initial copy number, the more template that is initially present the lower the cycle number where the fluorescence exceeds the threshold.
**Sentinel Molecular Beacon Core Reagent Kits**

For quantitative and qualitative PCR analysis

- Fast and convenient
- Exceptional sensitivity
- Great versatility
- Supports multiplexing and high-throughput formats

The Sentinel molecular beacon core reagent kits provide all the PCR and RT-PCR components needed for creating custom molecular beacon assays. Moreover, these kits are specifically designed for use with the Sentinel molecular beacon detection and expression analysis kits.

**PCR Core Reagent Kit**

- Detects a wide variety of DNA targets
- Wide linear range of amplification

The Sentinel molecular beacon PCR core reagent kit includes all the components necessary to carry out PCR amplification. This kit may be used in combination with Stratagene's molecular beacon probe kits or custom designed molecular beacons to amplify and detect a wide variety of DNA targets, including genomic DNA, cDNA and plasmid DNA.

This kit is sufficient for 200 reactions and includes a kit control. The kit is designed for use with the Sentinel molecular beacon detection kits, expression analysis kits and custom molecular beacon-based assays.

**Custom Molecular Beacon Synthesis**

Stratagene offers a custom synthesis service for molecular beacon fluorescent oligonucleotides.

- High-quality molecular beacons
- Multi-step purification process
- Quality assurance data sent with every probe
- Multiple fluorophores available (6-FAM, TET, HEX, Cy3, Cy5, TAMRA, Texas Red® and others by special order)
- Signal-to-noise and thermal denaturation profile available

These dual-labeled fluorescent oligonucleotides may be synthesized with a variety of dyes. They are shipped in a highly purified form with quality assurance data, including HPLC trace, molecular weight and quantity (O.D. units and nanomoles). When an order for a custom molecular beacon is combined with an order for a complementary nucleotide target, the signal-to-noise ratio and thermal denaturation profile for the beacon-target pair is also supplied.

To order a custom molecular beacon fluorescent oligonucleotide or for more information, please contact Stratagene’s Technical Support at 800-894-1304.

**Single-Tube RT-PCR Core Reagent Kit**

- Single-tube convenience and speed
- Analysis of gene expression accurate over a wide range of target inputs

The single-tube RT-PCR core reagent kit includes all the reagents necessary to quickly and conveniently carry out cDNA synthesis and PCR amplification in one tube for RNA analysis using molecular beacon detection. The convenient and fast single-tube format reduces the risk of carry-over contamination.

This kit is sufficient for 200 reactions and includes a kit control. The kit is designed for use with the Sentinel molecular beacon expression analysis kits and custom molecular beacon-based assays.

**RT-PCR Core Reagent Kit**

- Superior sensitivity
- Analysis of gene expression accurate over a wide range of target inputs

The RT-PCR core reagent kit is formatted for a traditional two-tube RT-PCR reaction and includes all the reagents necessary to carry out cDNA synthesis and PCR amplification for RNA analysis using molecular beacon detection. This format allows versatility and the possibility of running additional controls. The RT and PCR reactions are optimized to provide exceptional sensitivity.

This kit is sufficient for 200 reactions and includes a kit control. The kit is designed for use with the Sentinel molecular beacon expression analysis kits and custom molecular beacon-based assays.
Sentinel™ Molecular Beacon Detection Kits

For use as endogenous controls
- Compatible with human, rat and mouse templates
- Available for β-Actin or GAPDH
- Use either genomic DNA or cDNA templates
- Evaluate new molecular beacon systems
- Flexible temperature parameters
- Qualitative or quantitative PCR analysis

Sentinel™ molecular beacon detection kits provide positive endogenous control reactions for use in determining the presence of a specific nucleotide sequence. These kits may also be used to determine initial amount of target when using genomic DNA templates or to evaluate newly designed molecular beacon systems using this well-characterized control system.

These detection kits recognize PCR product generated from human, mouse and rat genomic DNA and cDNA templates. Each kit contains the specific molecular beacon and primer mix for 100 reactions, and includes a human genomic control template. These kits are designed for use with the Sentinel molecular beacon core reagent kits.

Great Sensitivity and Wide Linearity
The GAPDH detection kit demonstrates both great sensitivity and linearity using a serial dilution of human genomic DNA. The standard curve for this data set (not shown) is linear over 6 orders of magnitude with a regression correlation of 0.9975.

Sentinel™ Molecular Beacon Expression Analysis Kits

For quantitative gene expression analysis
- Highly specific for cDNA
- Sensitive RT-PCR analysis

Sentinel™ molecular beacon expression analysis kits are highly specific for cDNA due to the design of the molecular beacon and primer pair. This ensures highly accurate quantitative results. Stratagene offers a variety of expression analysis kits that include probes for control/housekeeping targets and targets useful for investigating gene expression.

Each kit contains the specific molecular beacon, primer mix for 100 reactions and a control template. These kits are specifically designed for use with the Sentinel molecular beacon core reagent kits.

Sentinel™ Molecular Beacon Allele Discrimination Kits

- Superior sensitivity

In a single PCR reaction, genotyping a specific polymorphism is performed by using two differentially labeled molecular beacons for wild-type and mutant alleles. The hairpin shape of the molecular beacon causes mismatched probe/target hybrids to easily dissociate at significantly lower temperatures than a perfectly complementary molecular beacon. The resulting increase in sensitivity makes molecular beacons the ideal choice for allele discrimination.

Each kit contains two allele-specific molecular beacons, sequence-matched DNA templates and PCR primers.

FluorTracker™ Fluorescence Reader

For endpoint PCR analysis
- Economical fluorometer for detecting presence of PCR products
- Optimized for PCR plates, tubes and strips
- Great sensitivity
- Easily accommodates simultaneous output of multiple thermal cyclers
- Separate thermal cycling and detection for high-throughput and economy
- Ideal for allele discrimination
- Detects a variety of fluorophores

The FluorTracker™ fluorescence reader provides fast, automated detection (qualitative endpoint assay) of PCR amplification products in conjunction with Sentinel™ molecular beacon kits. With this easy-to-use fluorometer, samples are simply transferred directly to the FluorTracker reader following thermal cycling for automatic measurement. Since the PCR samples are transferred without being opened, the risk of contamination by amplicons is minimized.

Software. The Windows®-based software interface provides precision control and maximum flexibility with point-and-click selection of filters, gain values, timing parameters and other functions. Microsoft® Excel performs data reduction, graphic analysis and unlimited customized calculations.

Detection of FAM and Texas Red Fluorophores

The FluorTracker fluorescence reader easily detects and discriminates multiple fluorophores. Each tube contains the FAM- and Texas Red-labeled molecular beacons and the target sequence as indicated. The y-axis represents the fluorescence signal of the sample divided by the signal obtained from the no-target control.

Sensitivity. Liquid light guide technology and a high-energy xenon flash lamp provide broad spectrum excitation. Combined with the system’s corresponding range of emission and excitation filters, this technology provides the sensitivity and versatility needed to collect data from experiments using a wide variety of fluorophores. An effective noise reduction system eliminates the influence of ambient light and PMT dark current to give the FluorTracker reader unsurpassed sensitivity.

Any microplate format. The customized plate holder accommodates PCR microplates, tubes and strips. By defining the locations of the corner wells, any microplate format may be read with minimal crosstalk.

Quantifying aP2 Gene Expression

The quantification of expression is easily determined between differentiated THP-1 cells, with and without stimulation (lanes 1-3 undifferentiated; lanes 4-6 PMA differentiated; lanes 1, 4 unstimulated; lanes 2, 5 LPS stimulated; lanes 3, 6 INF-γ stimulated), and is supported by observation of the RT-PCR products on an agarose gel.
A molecular beacon is a hybridization probe with a fluorophore and a non-fluorescent quencher molecule at opposite ends of an oligonucleotide. The ends of the oligonucleotide are designed to be complementary to each other and form a stem structure while the intervening loop is designed to be complementary to a sequence within the amplified product. When the molecular beacon is free in solution, it adopts a hairpin structure which brings the fluorophore and quencher sufficiently close to each other to allow quenching of the fluorophore. Molecular beacons whose loop sequence is complementary to the product amplified from the gene of interest are added to PCR reactions along with all the other PCR components.

When the complementary target is present, the molecular beacon spontaneously binds to this target. This binding causes a change in the conformation of the probe that places the fluorophore and quencher far enough apart that the fluorophore is no longer quenched and the molecular beacon fluoresces. The fluorescence of the molecular beacon is monitored during PCR or after cycling is complete. The amount of fluorescence indicates the amount of specific product present at that time. Real-time analysis monitors PCR at each cycle, allowing accurate quantitation. Endpoint analysis detects the presence of a particular sequence when PCR cycling is complete.

**PCR with Molecular Beacons**

- **Denaturation**: At denaturation temperatures, all nucleotide pairs are dissociated, and molecular beacons fluoresce brightly.
- **Annealing**: At annealing temperatures, appropriately designed molecular beacons hybridize to specific targets that are exact complements and the amount of fluorescence is proportional to the amount of product. If the target DNA sequence does not exactly match the molecular beacon sequence, even if only by a single base pair, hybridization and fluorescence will not occur. The thermodynamic properties of molecular beacons are such that they are more stable in the hairpin form than hybridized to a less than perfectly matched DNA sequence.
- **Extension**: At extension temperatures, the molecular beacons dissociate from the target, ensuring that the molecular beacon does not interfere with polymerization.
- **PCR Complete**: When PCR is completed, at room temperature or 4°C the molecular beacons remain bound to the specific target and fluorescence is proportional to the amount of specific amplicon present.
## Ordering Information

<table>
<thead>
<tr>
<th>Catalog No.</th>
<th>Description</th>
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<tbody>
<tr>
<td>600500</td>
<td>PCR Core Reagent Kit 200 rxns</td>
</tr>
<tr>
<td>600505</td>
<td>Single-Tube RT-PCR Core Reagent Kit 200 rxns</td>
</tr>
<tr>
<td>Coming Soon</td>
<td>RT-PCR Core Reagent Kit 200 rxns</td>
</tr>
</tbody>
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| 200570      | Sentinel™ Molecular Beacon Detection Kits |
| 200571      | |

| 200570      | Sentinel™ Molecular Beacon Detection Kits |
| 200573      | |

| 200575      | Sentinel™ Molecular Beacon Expression Analysis Kits |
| 200525      | |

| 200526      | Sentinel™ Molecular Beacon Allele Discrimination Kits |
| 200528      | |

| 200572      | FluorTracker™ Fluorescence Reader and Accessories |
| 200573      | |

| 200525      | |

| 200528      | |

| 401230      | FluorTracker™ Fluorescence Reader 120V |
| 401231      | 100V |
| 401232      | 230V |

| 401233      | Allele-Calling Software for use with FluorTracker™ Fluorescence Reader |
| 401234      | Coming Soon |

| 401235      | Custom Filter Sets for the FluorTracker™ Fluorescence Reader |
| 401236      | Call for Information |

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