SYBR® Green PCR Core Reagents Kit

- Real-Time Quantitation
- Fluorescent Detection of SYBR® Green I Dye
- Pre-Formulated Buffer

Benefits of Real-Time Quantitative PCR
Real-time quantitative PCR was first performed using the 5’ fluorogenic nuclease assay which uses a TaqMan® probe during the PCR amplification to provide added specificity. Now available is an additional chemistry, using SYBR® Green I dye, that can also provide real-time quantitative PCR information. Assays using the double-stranded DNA binding dye SYBR® Green I do not require a TaqMan® probe and provide additional experimental flexibility.

Assays Using SYBR Green I Dye
SYBR® Green I dye is believed to bind in the minor groove of double-stranded DNA and is fluorescent when bound. This binding characteristic is harnessed during PCR to monitor the process of amplification as PCR product is generated. An increasing amount of PCR product will result in an increase in SYBR® Green I dye fluorescence (Figure 1). As in the original fluorogenic 5’ nuclease assay, SYBR® Green I dye fluorescence is collected during the course of the reaction. The resulting real-time analysis enables more accurate quantitation of nucleic acids. Assays using SYBR® Green I dye also take advantage of normalization to the ROX internal passive reference on both the ABI Prism® 7700 and GeneAmp® 5700 Sequence Detection Systems. This minimizes well-to-well variability commonly caused by pipeting imprecision, thus ensuring more precise results (Figure 1).

Target Identification
Deciding which target is appropriate to develop into a routine assay may require some screening. Several targets may have to be tested on known samples to understand if the chosen target is appropriate. For this type of experiment (discovery), only a few runs are required. Although a TaqMan® probe provides an added level of specificity, the 5’ fluorogenic nuclease assay may not be necessary or cost effective to use when screening or discovering targets. SYBR® Green I dye can take some of the expense and time out of discovery assays when identifying an appropriate target. Once the target has been identified, the assay can be transformed quickly into a high-throughput 5’ nuclease assay that benefits from the added specificity of a TaqMan® probe.
Optimization Requirements

An assay using SYBR® Green I dye does not provide a second level of specificity, as is provided by the TaqMan® probe in a 5’ nuclease assay. The specificity of the assay is determined solely by the specificity of the primers in the given reaction conditions. Non-specific side reactions and primer-dimer artifacts must be minimized in order to obtain accurate results. This initial optimization requires dissociation curve (Figure 2) or gel analysis to determine if reactions are optimized sufficiently for specific target detection.

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<tr>
<th>Assay</th>
<th>TaqMan®</th>
<th>SYBR® Green I</th>
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<tbody>
<tr>
<td>Specificity</td>
<td>Primer &amp; Probe</td>
<td>Primer</td>
</tr>
<tr>
<td>Multiplex</td>
<td>Yes (7700 only)</td>
<td>No</td>
</tr>
<tr>
<td>Linear Dynamic Range</td>
<td>5 logs</td>
<td>5 logs</td>
</tr>
<tr>
<td>Recommended for:</td>
<td>• assays requiring validation</td>
<td>• discovery assays</td>
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<td></td>
<td>• repetitive assays</td>
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<tr>
<td></td>
<td>• assays requiring added specificity</td>
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Table 1. Assay comparison on the ABI Prism® 7700 and GeneAmp® 5700 Sequence Detection Systems.

Figure 2. Dissociation curve analysis performed on the GeneAmp® 5700 Sequence Detection System, after the completion of a real-time PCR run using SYBR Green I dye. The reduced melting temperature of the product formed in the No Template Control wells is characteristic of primer related non-specific amplification (primer-dimer).

Kit Components

- AmpliTaq Gold® DNA Polymerase
  AmpliTaq Gold® DNA Polymerase provides a better yield and a more robust reaction than AmpliTaq® DNA Polymerase. In addition, the hot-start property of AmpliTaq Gold helps to minimize non-specific amplification (including primer-dimer), which will be detected by SYBR Green I dye.
- AmpErase® UNG
  Prevents PCR product carryover contamination.
- Pre-Formulated Buffer
  Contains SYBR® Green I DNA binding dye as well as ROX passive reference 1 and is optimized for use in real-time analysis.
- dNTP Mix
- MgCl₂
  200 reactions (50 µL each)

Ordering Information

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<th>Description</th>
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<td>TaqMan® DNA Template Reagents (control)</td>
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<td>SDS Spectral Calibration Kit</td>
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<td>User Bulletin No. 4: Generating New Spectra Components</td>
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<tr>
<td>MicroAmp® Optical 96-Well Reaction Plates and Optical Caps</td>
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