Abstract
Sigma-Aldrich has developed quantitative PCR and RT-PCR products to meet a wide range of researcher needs. These products are designed to work with different detection chemistries and instruments for quantification.

Sigma-Aldrich Quantitative PCR ReadyMixes with SYBR Green

Sigma-Aldrich offers a specially formulated SYBR Green quantitative PCR ReadyMix for use on capillary based platforms, such as the Roche LightCycler™, while a second SYBR Green quantitative PCR ReadyMix is formulated for plate/tube real-time thermal cyclers. These SYBR Green quantitative PCR ReadyMixes are designed to provide high sensitivity, high specificity and lower CT values than master mixes from other suppliers.

Sigma-Aldrich Quantitative PCR ReadyMixes with Sequence-Specific Fluorescent Detection

Sigma-Aldrich offers two quantitative PCR ReadyMixes that do not contain a fluorescence detection method. These two mixes are compatible for use with many sequence-specific formats as well as nonspecific format. Dual-labeled probes, Molecular Beacons, or double stranded binding dyes such as SYBR Green I can all be individually optimized for use with these two ReadyMixes.

Table 1: Comparison of JumpStart Taq ReadyMix with dUTP with dye master mixes from three competitors.

<table>
<thead>
<tr>
<th>Vendor</th>
<th>Product Code</th>
<th>Efficiency</th>
<th>Sensitivity (pg/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplier A</td>
<td>-0.500</td>
<td>1.000</td>
<td>0.100</td>
</tr>
<tr>
<td>Supplier B</td>
<td>-0.500</td>
<td>1.000</td>
<td>0.100</td>
</tr>
<tr>
<td>Supplier C</td>
<td>-0.500</td>
<td>1.000</td>
<td>0.100</td>
</tr>
</tbody>
</table>

Conclusions

JumpStart Taq ReadyMix with dUTP gives comparable performance results when compared with three leading competitors on both capillary and non-capillary formats.


SYBR Green Quantitative PCR/RT-PCR Kit

The SYBR Green quantitative RT-PCR kit combines SYBR™ RT, an enhanced avian reverse transcriptase, JumpStart Taq DNA polymerase and SYBR Green I fluorescent dye in a one step RT-PCR kit designed for measurement of gene expression. SYBR™ RT has the ability to transcribe through difficult secondary structure at elevated temperatures (up to 65°C). JumpStart Taq DNA polymerase uses JumpStart Taq anti- body to inactivate the enzyme below 70°C, preventing primer-dimer and non-specific product formation. Since SYBR Green I dye will detect all nonspecific quantitative RT-PCR product formation, well-designed primers are recommended for this system to ensure the highest possible specificity.

The kit has been optimized for use with both plate/tube real-time instruments and with the Roche LightCycler capillary instrument. A reference dye is provided in a separate vial to be used with ABI Detection Systems. A vial of 25 mM MgCl₂ is also provided for further optimization.

Quantitative SYBR Green one-step RT-PCR was performed on human total RNA from cell line HeLa-S3. The total RNA was DNase treated using Sigma’s DNase kit (Product Code AMP-D1) and diluted 10-fold in subsequent capillaries; concentrations were 500 ng, 50 ng, 5 ng, 500 pg, 50 pg, 5 pg. Specific primers for a β-actin gene were used. An additional 15 minute incubation was added to the performance enhancements of JumpStart Taq antibody for hot start PCR in a convenient, easy-to-use 2X concentrate.

SYBR Green JumpStart Taq ReadyMix, Product Code S 4438, is recommended for use in ABI Detection Systems along with a vial of 25 mM MgCl₂ for further optimization.

Fluorescence (F1) values are plotted against cycle number (Cycle) for each primer set in Figure 3. SYBR Green JumpStart Taq ReadyMix has a linear response over seven orders of magnitude (Figure 4).

SYBR Green JumpStart Taq ReadyMix, Product Code S 1816, is specially designed for use in capillary-based instruments, such as the Roche LightCycler real-time thermal cycle. A vial of 25 mM MgCl₂ is provided for further optimization.

Quantitative PCR was performed using human genomic DNA. The template was diluted 10-fold in subsequent wells; concentrations were 10 ng, 1 ng, 0.1 ng, and 0.01 ng. A TaqMan® probe and primer specific for a 250 bp PCR product of the β-actin gene were used. An additional 15 minute incubation was added to the cycle program for the master mix from Supplier Q and an additional 15 minute incubation was added to the cycle program for the master mix from Supplier R for activation of JumpStart Taq antibody with dUTP (for a higher efficiency than the master mix from Supplier S.)

JumpStart Taq ReadyMix with dUTP, Product Code D 9191, incorporates dUTP in place of dTTP to facilitate carryover prevention. This dUTP containing PCR ReadyMix is formulated for use on both plate/tube and capillary-based real-time instruments.

Fluorescence (F1) values are plotted against cycle number (Cycle) for each primer set in Figure 7. SYBR Green JumpStart Taq ReadyMix with Capillary Formulation has a linear response over six orders of magnitude with high sensitivity (Figure 8).

Figure 1: JumpStart Taq ReadyMix with dUTP, Product Code D 9191, incorporates dUTP in place of dTTP to facilitate carryover prevention. This dUTP containing PCR ReadyMix is formulated for use on both plate/tube and capillary-based real-time instruments.