foodproof® *Saccharomyces cerevisiae* var. *diastaticus*
Detection Kit
- Hybridization Probes (LC 1.x, 2.0) -

Order No. R 300 26

**Quick Reference Procedure**
Version 2, March 2012

**A. LightCycler® Carousel-Based System Protocol**
The amplification and Melting Curve generation with the LightCycler® 1.x Instrument or LightCycler® 2.0 Instrument is carried out according to the following temperature-time-program (for details on how to program the experimental protocol, see the LightCycler Instrument® Operator's Manual):

### Pre-incubation

<table>
<thead>
<tr>
<th>Programs/Cycle Program Data</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles</td>
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</tr>
<tr>
<td>Analysis Mode</td>
<td>None</td>
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<table>
<thead>
<tr>
<th>Temperature Targets</th>
<th>Segment 1</th>
<th>Segment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target/Target Temperature [°C]</td>
<td>37</td>
<td>95</td>
</tr>
<tr>
<td>Hold/Incubation Time [h:min:s]</td>
<td>00:02:00</td>
<td>00:15:00</td>
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<tr>
<td>Ramp Rate/Target Temperature Transition Rate [°C/s]</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Secondary Target Temperature [°C]</td>
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</tr>
<tr>
<td>Step Size [°C]</td>
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<tr>
<td>Step Delay [cycles]</td>
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### Amplification

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<th>Temperature Targets</th>
<th>Segment 1</th>
<th>Segment 2</th>
<th>Segment 3</th>
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</thead>
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<td>Target/Target Temperature [°C]</td>
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<tr>
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<td>00:00:30</td>
<td>00:00:15</td>
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<tr>
<td>Ramp Rate/Target Temperature Transition Rate [°C/s]</td>
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<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Secondary Target Temperature [°C]</td>
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<td>Step Size [°C]</td>
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<tr>
<td>Step Delay [cycles]</td>
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### Melting Curve Analysis

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<td>Analysis Mode</td>
<td>Melting Curves</td>
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<table>
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<th>Temperature Targets</th>
<th>Segment 1</th>
<th>Segment 2</th>
<th>Segment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target/Target Temperature [°C]</td>
<td>95</td>
<td>38</td>
<td>80</td>
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<tr>
<td>Hold/Incubation Time [h:min:s]</td>
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<td>00:01:00</td>
<td>00:00:00</td>
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<td>Ramp Rate/Target Temperature Transition Rate [°C/s]</td>
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<tr>
<td>Secondary Target Temperature [°C]</td>
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<tr>
<td>Step Size [°C]</td>
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<td>0.0</td>
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<td>Step Delay [cycles]</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>Acquisition Mode</td>
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</table>

Continued next page
Cooling Programs/Cycle Program Data

<table>
<thead>
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<th>Analysis Mode</th>
<th>Temperature Targets</th>
<th>Acquisition Mode</th>
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<tbody>
<tr>
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<td>Cycles 1</td>
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<td>Analysis Mode</td>
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<td>Target/Target Temperature [°C]</td>
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</tr>
<tr>
<td>Hold/Incubation Time [h:min:s]</td>
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</tr>
<tr>
<td>Ramp Rate/Temperature Transition Rate [°C/s]</td>
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<td>Sec Target/Secondary Target Temperature [°C]</td>
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<td>Step Size [°C]</td>
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<tr>
<td>Step Delay [cycles]</td>
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</tr>
<tr>
<td>Acquisition Mode</td>
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</table>

Standard Protocol

It is recommended to use filter tips!

1. Determine the total number of PCR reactions (sample and control reactions) and calculate the required volumes. In addition to the number of samples, two control reactions (negative control and positive control) should be included in each LightCycler® run. Due to pipetting errors it is recommended to prepare the volumes of Master Mix, Enzyme Solution and Internal Control for 1 – 2 reactions additionally. An example is given in Table 1.

*Note: The volumes indicated below are based on a single 20 µl standard reaction. Prepare the PCR mix by multiplying the amount in the “Volume” column by the number of reactions to be cycled plus one or two additional reactions.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>foodproof Saccharomyces cerevisiae var. diastaticus Master Mix, (vial 1, yellow cap)</td>
<td>13 µl</td>
</tr>
<tr>
<td>foodproof Saccharomyces cerevisiae var. diastaticus Enzyme Solution (vial 2, red cap)</td>
<td>1.0 µl</td>
</tr>
<tr>
<td>foodproof Saccharomyces cerevisiae var. diastaticus Internal Control (vial 3, white cap)</td>
<td>1.0 µl</td>
</tr>
</tbody>
</table>

- Total volume: 15.0 µl

2. Depending on the total number of reactions, place LightCycler® Capillaries in the centrifuge adapters.
3. Thaw, mix gently (do not vortex!) but thoroughly and centrifuge all reagents.
4. Transfer the calculated volume of Master Mix into a new sterile reaction tube. Add the corresponding volume of Enzyme Solution and Internal Control to the Master Mix. Mix thoroughly by pipetting.
5. Transfer 15.0 µl of PCR mix including Internal Control into all prepared capillaries.
6. For the samples of interest, add up to 5 µl sample DNA (if less than 5 µl add H₂O to 5 µl) to a capillary. Seal the capillaries with a stopper.
7. For the negative control, add 5 µl PCR-grade H₂O (vial 4, colorless cap). Seal the capillary with a stopper.
8. For the positive control, add 5 µl foodproof Saccharomyces cerevisiae var. diastaticus Control Template (vial 4, purple cap). Seal the capillary with a stopper.
9. Place capillaries into adapters and centrifuge at 700 x g for 5 seconds (3,000 rpm in a standard bench-top microcentrifuge).
10. Place the capillaries in the rotor of the LightCycler®-Instrument.
11. Start the PCR run.

*Note: For further information please refer to: www.bc-diagnostics.com/?cid=1236596070&lang=1