

# LightCycler™ -Primer Set

Ready-to-use amplification primer mix for RT-PCR using the LightCycler™ Instrument

## Human DPYD (dihydropyrimidine dehydrogenase)

Kit for 96 reactions

Lot # 260105 Exp. 26.01.2007

**Note:** After Thawing keep on ice!

Store the kit at -20°C

|                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                     |                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                   |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1. Kit Contents                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                     |                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                   |
| <b>caution</b>                                                                                                                                                                                                                                                                                 | After Thawing keep on ice!                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                     | <b>Sample material</b>                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                   |
| <b>Kit contents</b>                                                                                                                                                                                                                                                                            | <b>Vial</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | <b>Label</b>                        | <b>Sample Preparation</b>                                                                                                                              |                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                                                                                                                                                                                                | 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | DPYD<br>Primer mix<br>Yellow cap    |                                                                                                                                                        | <p>Reliable and reproducible results are achieved with 1 µg total RNA isolated with the HighPure total RNA Isolation Kit (Roche) reverse transcribed with the 1<sup>st</sup> Strand cDNA Synthesis Kit (AMV) (Roche).<br/> <b>! The resulting cDNA has to be diluted to a final volume of 200-500 µl with PCR-grade water</b></p> |
|                                                                                                                                                                                                                                                                                                | 2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Standard<br>Red cap                 |                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                                                                                                                                                                                                | 3                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Standard<br>Stabilizer<br>Green cap |                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                                                                                                                                                                                                | 4                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Control cDNA<br>Blue cap            |                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                   |
| 5                                                                                                                                                                                                                                                                                              | H <sub>2</sub> O, sterile,<br>PCR grade<br>White cap                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                     |                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                   |
| <b>Content and use</b>                                                                                                                                                                                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                     | <b>Application</b>                                                                                                                                     |                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                                                                                                                                                                                                | <ul style="list-style-type: none"> <li>• 200 µl ready-to-use primer mix for target specific amplification using the LightCycler™ FastStart Master Sybr Green I contains optimal MgCl<sub>2</sub> concentration and amplification primer pair</li> <li>• 60 µl amplification standard for approximately 27000 copies/µl of DPYD cDNA</li> <li>• 300 µl Solution for dilution of standard</li> <li>• 50 µl contains a cDNA mix from several human hematopoietic cell lines</li> <li>• 1 ml to adjust the final reaction volume</li> </ul> |                                     | Quantitative evaluation of gene expression in human cells and tissue                                                                                   |                                                                                                                                                                                                                                                                                                                                   |
| <b>Additional equipment and reagents required</b>                                                                                                                                                                                                                                              | <p>1<sup>st</sup> Strand cDNA Synthesis Kit for RT-PCR (Roche Cat. # 1 483 188)<br/>           LightCycler™ FastStart Master SybrGreen I (Roche Cat. # 3 003 230)<br/>           LightCycler™ Instrument (Roche Cat. # 2 011 468)<br/>           LightCycler™ Primer Set Housekeeping genes (Search GmbH)</p>                                                                                                                                                                                                                           |                                     | <b>Assay time</b>                                                                                                                                      |                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                     | Set up the PCR amplification <b>15 min</b><br>LightCycler™ PCR run <b>50 min</b>                                                                       |                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                     | <b>Number of tests</b>                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                     | The Kit is designed for 96 Reactions                                                                                                                   |                                                                                                                                                                                                                                                                                                                                   |
| 2. Introduction                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                     | <b>Quality Control</b>                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                   |
| <p>The LightCycler™-Primer Set allows to perform quantitative RT-PCR using the LightCycler™ instrument. An optimized primer pair has been selected for specific amplification of targets. The amplicon is detected by fluorescence using the double-stranded DNA binding dye Sybr®Green I.</p> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                     | <p>The LightCycler™-Primer Set is tested using the LightCycler™ FastStart Master Sybr®Green I according to the protocol described below.</p>           |                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                     | <b>Kit storage/ stability</b>                                                                                                                          |                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                     | The unopened kit is stable at -20°C 24 month from date of QC-release.                                                                                  |                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                     | <b>Specificity</b>                                                                                                                                     |                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                     | The LightCycler™-Primer Set "DPYD" is specific for the sequence of human DPYD and does not detect genomic DPYD specific sequences if used as directed. |                                                                                                                                                                                                                                                                                                                                   |

### 3. Procedure

|                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |              |             |                                                                     |                  |                                     |           |
|---------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|-------------|---------------------------------------------------------------------|------------------|-------------------------------------|-----------|
| <b>Introduction</b>                                                 | A fragment of the human DPYD cDNA sequence is amplified and monitored with the dsDNA specific Sybr <sup>®</sup> Green I dye                                                                                                                                                                                                                                                                                                                                                                                                     |              |             |                                                                     |                  |                                     |           |
| <b>Additional reagents required</b>                                 | LightCycler <sup>™</sup> FastStart Master Sybr <sup>®</sup> Green I (Cat.# 3 003 230)                                                                                                                                                                                                                                                                                                                                                                                                                                           |              |             |                                                                     |                  |                                     |           |
| <b>Thawing the solutions</b>                                        | Thaw the following reagents, mix gently, and store on ice:<br><br><table border="0"> <tr> <td style="border-bottom: 1px solid black;">From the ...</td> <td style="border-bottom: 1px solid black;">Thaw the...</td> </tr> <tr> <td>LightCycler<sup>™</sup> FastStart Master Sybr<sup>®</sup>Green I</td> <td>vial <b>1a/b</b></td> </tr> <tr> <td>LightCycler<sup>™</sup> Primer Set</td> <td>all tubes</td> </tr> </table> <p><b>It is recommended to define the experimental protocol before preparing the solutions</b></p> | From the ... | Thaw the... | LightCycler <sup>™</sup> FastStart Master Sybr <sup>®</sup> Green I | vial <b>1a/b</b> | LightCycler <sup>™</sup> Primer Set | all tubes |
| From the ...                                                        | Thaw the...                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |              |             |                                                                     |                  |                                     |           |
| LightCycler <sup>™</sup> FastStart Master Sybr <sup>®</sup> Green I | vial <b>1a/b</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |              |             |                                                                     |                  |                                     |           |
| LightCycler <sup>™</sup> Primer Set                                 | all tubes                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |              |             |                                                                     |                  |                                     |           |
| <b>Experimental Protocol</b>                                        | The described protocol consists of four programs. <ul style="list-style-type: none"> <li>• Program 1: Denaturation of the template and activation of the polymerase</li> <li>• Program 2: Amplification of the target</li> <li>• Program 3: Melting curve analysis for product control</li> <li>• Program 4: Cooling the rotor and thermal chamber</li> </ul>                                                                                                                                                                   |              |             |                                                                     |                  |                                     |           |

### Denaturation

| Parameter                    | Value     |
|------------------------------|-----------|
| Cycles                       | 1         |
| Type                         | Regular   |
| Temp. Targets                | Segment 1 |
| Target Temperature           | 95        |
| Incubation time (h:min:s)    | 10:00     |
| Temp. Transition Rate (°C/s) | 20        |
| Secondary Target Temp.       | 0         |
| Step Size                    | 0         |
| Step Delay                   | 0         |
| Aquisition Mode              | None      |

### Amplification

| Parameter                    | Value          |       |        |
|------------------------------|----------------|-------|--------|
| Cycles                       | 35             |       |        |
| Type                         | Quantification |       |        |
| Temp. Targets                | Seg.1          | Seg.2 | Seg.3  |
| Target Temperature           | 95             | 68    | 72     |
| Incubation time (h:min:s)    | 10             | 10    | 16     |
| Temp. Transition Rate (°C/s) | 20             | 20    | 20     |
| Secondary Target Temp.       | 0              | 58    | 0      |
| Step Size                    | 0              | 0.5   | 0      |
| Step Delay                   | 0              | 1     | 0      |
| Aquisition Mode              | None           | None  | Single |
| Gains                        | F1 = 5         |       |        |

### Melting Curve Analysis

| Parameter                    | Value         |        |       |
|------------------------------|---------------|--------|-------|
| Cycles                       | 1             |        |       |
| Type                         | Melting Curve |        |       |
| Temp. Targets                | Seg.1         | Seg. 2 | Seg.3 |
| Target Temperature           | 95            | 58     | 95    |
| Incubation time (h:min:s)    | 0             | 10     | 0     |
| Temp. Transition Rate (°C/s) | 20            | 20     | 0.1   |
| Secondary Target Temp.       | 0             | 0      | 0     |
| Step Size                    | 0             | 0      | 0     |
| Step Delay                   | 0             | 0      | 0     |
| Aquisition Mode              | None          | None   | Cont. |

### Cooling

| Parameter                    | Value     |
|------------------------------|-----------|
| Cycles                       | 1         |
| Type                         | Regular   |
| Temp. Targets                | Segment 1 |
| Target Temperature           | 40        |
| Incubation time (h:min:s)    | 30        |
| Temp. Transition Rate (°C/s) | 20        |
| Secondary Target Temp.       | 0         |
| Step Size                    | 0         |
| Step Delay                   | 0         |
| Aquisition Mode              | None      |

|                                      |                                                                                                                                                                                                                                                                                                                                                                          |
|--------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Preparation of the master mix</b> | Depending on the total number of reactions place LightCycler™ capillaries in pre-cooled centrifuge adaptors.<br>It is recommended to use electronic pipettors with high quality tips (low volume retention). Prepare a master mix by multiplying the amount in the “Volume” column by the number of reactions to be analyzed, plus five additional reactions (Standard). |
|--------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

| Step                                                      | Action                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |           |      |                              |           |                                      |           |                                                           |           |                     |                             |
|-----------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|------|------------------------------|-----------|--------------------------------------|-----------|-----------------------------------------------------------|-----------|---------------------|-----------------------------|
| <b>1</b>                                                  | Prepare a fresh dilution series of the standard using the standard stabilizer solution<br>1:10 = 2700 copies/ $\mu$ l<br>1:100 = 270 copies/ $\mu$ l<br>1:1000 = 27 copies/ $\mu$ l                                                                                                                                                                                                                                                                                                                                                                                                                           |           |      |                              |           |                                      |           |                                                           |           |                     |                             |
| <b>2</b>                                                  | In a 1.5 ml light protected reaction tube on ice, add the following components in the order mentioned below: <table border="1" data-bbox="188 981 699 1176"> <thead> <tr> <th>Component</th> <th>Vol.</th> </tr> </thead> <tbody> <tr> <td>H<sub>2</sub>O (white cap)</td> <td>6 <math>\mu</math>l</td> </tr> <tr> <td>LightCycler™ Primer Set (yellow cap)</td> <td>2 <math>\mu</math>l</td> </tr> <tr> <td>LightCycler™ FastStart DNA Master Sybr®Green I (premixed)</td> <td>2 <math>\mu</math>l</td> </tr> <tr> <td><b>Total Volume</b></td> <td><b>10 <math>\mu</math>l</b></td> </tr> </tbody> </table> | Component | Vol. | H <sub>2</sub> O (white cap) | 6 $\mu$ l | LightCycler™ Primer Set (yellow cap) | 2 $\mu$ l | LightCycler™ FastStart DNA Master Sybr®Green I (premixed) | 2 $\mu$ l | <b>Total Volume</b> | <b>10 <math>\mu</math>l</b> |
| Component                                                 | Vol.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |           |      |                              |           |                                      |           |                                                           |           |                     |                             |
| H <sub>2</sub> O (white cap)                              | 6 $\mu$ l                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |           |      |                              |           |                                      |           |                                                           |           |                     |                             |
| LightCycler™ Primer Set (yellow cap)                      | 2 $\mu$ l                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |           |      |                              |           |                                      |           |                                                           |           |                     |                             |
| LightCycler™ FastStart DNA Master Sybr®Green I (premixed) | 2 $\mu$ l                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |           |      |                              |           |                                      |           |                                                           |           |                     |                             |
| <b>Total Volume</b>                                       | <b>10 <math>\mu</math>l</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |           |      |                              |           |                                      |           |                                                           |           |                     |                             |
| <b>3</b>                                                  | <ul style="list-style-type: none"> <li>Pipet <b>10 <math>\mu</math>l</b> PCR mix into the pre-cooled LightCycler™ capillary</li> <li>Add <b>10 <math>\mu</math>l</b> of cDNA template</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                              |           |      |                              |           |                                      |           |                                                           |           |                     |                             |
| <b>4</b>                                                  | <ul style="list-style-type: none"> <li>Pipet <b>10 <math>\mu</math>l</b> of PCR mix into 4 pre-cooled LightCycler™ capillaries</li> <li>Add <b>10 <math>\mu</math>l</b> of undiluted and of the freshly diluted standards into each capillary</li> </ul>                                                                                                                                                                                                                                                                                                                                                      |           |      |                              |           |                                      |           |                                                           |           |                     |                             |
| <b>5</b>                                                  | Seal each capillary with a stopper and place the adaptors, containing the capillary, into a benchtop microcentrifuge. Centrifuge at 2000 rpm for 30 s.                                                                                                                                                                                                                                                                                                                                                                                                                                                        |           |      |                              |           |                                      |           |                                                           |           |                     |                             |
| <b>6</b>                                                  | Place capillaries in the rotor of the LightCycler™ Instrument.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |           |      |                              |           |                                      |           |                                                           |           |                     |                             |
| <b>7</b>                                                  | Cycle the samples as described above                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |           |      |                              |           |                                      |           |                                                           |           |                     |                             |

| Typical results     |                                                                                                                                                                                                                                                                             |
|---------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Introduction</b> | The analysis of the obtained data is divided into two parts: <ul style="list-style-type: none"> <li>Part 1: Use of the quantification program, followed by</li> <li>Part 2: Specificity control of the amplification reaction by using the melting curve program</li> </ul> |

|                               |                                                                                                                                                                                                                                                                                                                                                                                     |
|-------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Quantification program</b> | The attached amplification curves in the QC sheet were obtained by performing the described procedure with the enclosed standards and control cDNA. The fluorescence values versus cycle number are displayed.<br>The enclosed control cDNA contains approximately 150 copies per $\mu$ l of DPYD specific cDNA                                                                     |
| <b>Melting curve program</b>  | Assess the specificity of the amplified PCR product by performing a melting curve analysis.<br>The resulting melting curves allow discrimination between specific and unspecific product<br>The attached melting curves in the QC sheet display the amplification of the control cDNA. As a control for the specificity, 5ng of human genomic DNA was amplified in this experiment. |



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