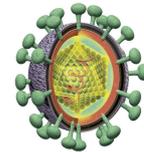


1. LIPSGENE® – PATHOGEN DETECTION



Application

Depending on the target, the LIPSGENE® Kits are intended for either real-time PCR quantification or qualitative analysis of viral or bacterial DNA/RNA in clinical samples, e.g. EDTA plasma, serum, sputum etc. Concerning some targets, a novel homogenous assay allows, besides quantification of distinct targets, also detection of known polymorphisms, performing genotyping at the same time. Several kit versions for distinct real-time PCR platforms are available depending on the kind of PCR vessel used, e.g. for instruments supporting 0.2 ml regular profile 8-well/strip PCR tubes and cap strips (RP; e.g. ABI PRISM® 7000/7300/7700 SDS [Applied Biosystems/Life Technologies], iCycler IQ™; IQ5 [Bio-Rad], MX3000P, Mx3005P [Stratagene]; Mastercycler® ep realplex [Eppendorf]); single 0.2 ml PCR Tubes with attached cap (ST; e.g. Rotor-Gene™ 3000/6000, Rotor-Gene Q (Qiagen); LineGene K (Bioer); 0.1 ml low profile 8-well/strip PCR tubes and cap strips (LP; e.g. , 7500 Fast [Applied Biosystems/Life Technologies], MiniOpticon™, CFX96 [Bio-Rad]; 20 µl LightCycler glass capillaries (LC; e.g. LightCycler 2.x); 25 µl SmartCycler tubes (SC; e.g. SmartCycler® [Cepheid]); and 20 µl DX-12 reaction tubes (SP; e.g. Spartan Dx-12 [Spartan Bioscience Inc.]).

The kits are not intended for screening of blood or blood products.

Explanation of the kits

The LIPSGENE® Kits are real time PCR amplification tests for quantification and/or qualitative analysis of pathogen derived DNA or RNA, in clinical samples. The kits consist of two boxes. Box 1, shipped at room temperature, includes lyophilized oligonucleotide mix (optimized compositions of primers, probes, dNTPs and carrier nucleic acids), target-specific standard tubes, stably coated with given amounts of synthetic standards DNA or RNA, PCR plastic ware.

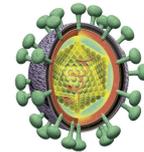
Box 2, provided on dry ice in a separate polystyrene box, contains the enzymes and the corresponding buffers. A separate bag includes 2.0 ml safe-lock tubes that contain stabilized internal DNA or RNA control. All goods have to be stored at -20°C immediately upon arrival to guarantee the printed shelf-life.

For sample preparation, several kits are recommended, e.g. the "LIPSPREP® Virus Purification Kit". Pathogen DNA or RNA extraction must be performed with the respective starting sample volume strictly according to manufacturer's instructions.

Internal Control

The LIPSGENE® Pathogen Detection/Quantification Kits are provided together with stabilized synthetic internal DNA control (DC) or RNA control (RC). Controls are contained in the respective 2.0 ml-tubes labelled with either the "DC" abbreviation or the "RC" one. Amplification of target-containing specimens and standards on one hand, and of an internal control on the other hand, requires measurement at different wavelengths due to probes labelled with diverse fluorescence reporter dyes. In most of the protocols, e.g. kits for use with iCycler IQ™; IQ5 (Bio-Rad); MX3000P, Mx3005P (Stratagene); Mastercycler® ep realplex (Eppendorf), Rotor-Gene™ 3000/6000, Rotor-Gene Q (Qiagen); LineGene K (Bioer), MiniOpticon™, CFX96™ (Bio-Rad); LightCycler™ 480, LightCycler™ 96, LightCycler™ 1.x/2.x (Roche); SmartCycler® (Cepheid); and

1. LIPSGENE[®] – PATHOGEN DETECTION



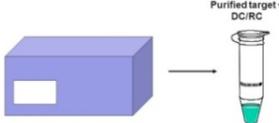
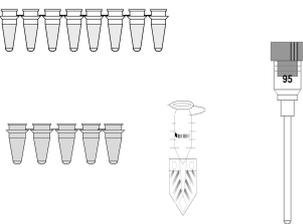
Spartan Dx-12 (Spartan Bioscience Inc.) the measuring channels are denoted as FAM (Green) and ROX (Orange), respectively.

Kit versions for use with ABI PRISM[®] 7000/7300/7700 SDS, 7500 Fast (Applied Biosystems/Life Technologies) require detection in both the FAM (Green) and JOE/VIC (Yellow) channels.

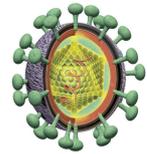
Applying the DC containing extraction tubes together with the DNA extraction kit and the RC containing extraction tubes together with the RNA extraction kit of choice, always allows to control for extraction yield and inhibitor load and also makes possible to judge the efficiencies of nucleic acids extraction and subsequent PCR amplification. False-negatives due to failed extraction or excess of inhibitors in the sample may be excluded when getting positive amplification results for the internal control.

In case of using e.g. the QIAamp DSP Virus Kit replace the original lysis tubes labelled with "LT" by the sample preparation tubes contained in a separate bag.

To consider the purification successful, the C_T value of the DC purified together with a sample that is negative for the target should be in a defined range. The kit manuals contain detailed information what C_T range is accepted concerning the respective real time PCR instruments.

| | |
|---|---|
| <p>1. Perform sample lysis within the DC or the RC tubes.</p> |  <p>2.0 mL Safe-lock tubes (separate bag) Use lysis solutions of the respective purification kit.</p> |
| <p>2. Perform nucleic acids extraction using purification kit of choice.</p> |  <p>Perform extraction according to recommendations of the manufacturer of the purification kit.</p> |
| <p>3. Prepare 5x oligonucleotide mix.</p> |  <p>Add 35 μL PCR grade water, incubate at 37°C for 10 min, mix by vortexing for 3 sec.</p>  5 sec, 10,000 g |
| <p>4. Prepare and aliquote 1x master mix to tubes, add sample</p> |  <p>Add 20 μL of 1x master mix to all sample tubes and quantification standard tubes.</p> <p>Add 5 μL of extracted sample to sample tubes.</p> <p>Add 5 μL PCR grade water to sample tubes which serves as NTC and to quantitation standard tubes.</p>  5 sec, 1,500 g |

1. LIPSGENE[®] – PATHOGEN DETECTION



| <p>5. Cover tubes, set up and perform the real-time PCR run</p> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|--|---|---------------------|---------|--------|-------|---------|-------------|------------------------|-----------------------|----------|-------------|--|--|------|-------|--------|---------|--------|----|---------|-------------|------------------------|-----------------------|---------|-------------|-----|-----|--|-----|--------|-------|-------|-------|-----------|-------|----------|----------|-----|-----|--|-----|--------|-------|-------|-------|-----------|-------|----------|----------|-----|-----|--|-----|--------|-------|-------|-------|-----------|-------|----------|----------|-----|-----|--|-----|--------|-------|-------|-------|-----------|-------|----------|----------|-----|-----|--|-----|--------|-------|-------|-------|-----------|-------|----------|----------|-----|-----|--|------|----|-------|-------|-------|-----------|-------|----------|----------|-----|-----|--|------|----|-------|-------|-------|-----------|-------|----------|----------|-----|-----|--|------|----|-------|-------|-------|-----------|-------|----------|----------|-----|-----|--|------|----|-------|-------|-------|-----------|-------|----------|----------|
| <p>6. Analyze runs</p> | <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>Standard/target growth curves</p> </div> <div style="text-align: center;"> <p>RC growth curves</p> </div> </div> <div style="text-align: center; margin-top: 10px;"> <p>Standard reference curve</p> </div> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <p>7. Report: quantitative results</p> | <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th colspan="12">Quantification Data</th> </tr> <tr> <th>Well</th> <th>Floor</th> <th>Target</th> <th>Content</th> <th>Sample</th> <th>Cq</th> <th>Cq Mean</th> <th>Cq Std. Dev</th> <th>Starting Quantity (SQ)</th> <th>Log Starting Quantity</th> <th>SQ Mean</th> <th>SQ Std. Dev</th> </tr> </thead> <tbody> <tr> <td>A01</td> <td>FAM</td> <td></td> <td>Std</td> <td>HCV/IC</td> <td>18.88</td> <td>18.88</td> <td>0,000</td> <td>1,000E+06</td> <td>6,000</td> <td>1,00E+06</td> <td>0,00E+00</td> </tr> <tr> <td>A02</td> <td>FAM</td> <td></td> <td>Std</td> <td>HCV/IC</td> <td>24.28</td> <td>24.28</td> <td>0,000</td> <td>1,000E+04</td> <td>4,000</td> <td>1,00E+04</td> <td>0,00E+00</td> </tr> <tr> <td>A03</td> <td>FAM</td> <td></td> <td>Std</td> <td>HCV/IC</td> <td>28.19</td> <td>28.19</td> <td>0,000</td> <td>1,000E+03</td> <td>3,000</td> <td>1,00E+03</td> <td>0,00E+00</td> </tr> <tr> <td>A04</td> <td>FAM</td> <td></td> <td>Std</td> <td>HCV/IC</td> <td>31.95</td> <td>31.95</td> <td>0,000</td> <td>5,000E+01</td> <td>1,699</td> <td>5,00E+01</td> <td>0,00E+00</td> </tr> <tr> <td>A05</td> <td>FAM</td> <td></td> <td>Std</td> <td>HCV/IC</td> <td>34.39</td> <td>34.39</td> <td>0,000</td> <td>1,000E+01</td> <td>1,000</td> <td>1,00E+01</td> <td>0,00E+00</td> </tr> <tr> <td>B01</td> <td>FAM</td> <td></td> <td>Unkn</td> <td>1a</td> <td>33.33</td> <td>33.33</td> <td>0,000</td> <td>1,922E+01</td> <td>1,284</td> <td>1,92E+01</td> <td>0,00E+00</td> </tr> <tr> <td>B02</td> <td>FAM</td> <td></td> <td>Unkn</td> <td>1a</td> <td>32.61</td> <td>32.61</td> <td>0,000</td> <td>3,282E+01</td> <td>1,516</td> <td>3,28E+01</td> <td>0,00E+00</td> </tr> <tr> <td>B03</td> <td>FAM</td> <td></td> <td>Unkn</td> <td>1b</td> <td>32.53</td> <td>32.53</td> <td>0,000</td> <td>3,474E+01</td> <td>1,541</td> <td>3,47E+01</td> <td>0,00E+00</td> </tr> <tr> <td>B04</td> <td>FAM</td> <td></td> <td>Unkn</td> <td>1b</td> <td>31.75</td> <td>31.75</td> <td>0,000</td> <td>6,158E+01</td> <td>1,789</td> <td>6,16E+01</td> <td>0,00E+00</td> </tr> </tbody> </table> | Quantification Data | | | | | | | | | | | | Well | Floor | Target | Content | Sample | Cq | Cq Mean | Cq Std. Dev | Starting Quantity (SQ) | Log Starting Quantity | SQ Mean | SQ Std. Dev | A01 | FAM | | Std | HCV/IC | 18.88 | 18.88 | 0,000 | 1,000E+06 | 6,000 | 1,00E+06 | 0,00E+00 | A02 | FAM | | Std | HCV/IC | 24.28 | 24.28 | 0,000 | 1,000E+04 | 4,000 | 1,00E+04 | 0,00E+00 | A03 | FAM | | Std | HCV/IC | 28.19 | 28.19 | 0,000 | 1,000E+03 | 3,000 | 1,00E+03 | 0,00E+00 | A04 | FAM | | Std | HCV/IC | 31.95 | 31.95 | 0,000 | 5,000E+01 | 1,699 | 5,00E+01 | 0,00E+00 | A05 | FAM | | Std | HCV/IC | 34.39 | 34.39 | 0,000 | 1,000E+01 | 1,000 | 1,00E+01 | 0,00E+00 | B01 | FAM | | Unkn | 1a | 33.33 | 33.33 | 0,000 | 1,922E+01 | 1,284 | 1,92E+01 | 0,00E+00 | B02 | FAM | | Unkn | 1a | 32.61 | 32.61 | 0,000 | 3,282E+01 | 1,516 | 3,28E+01 | 0,00E+00 | B03 | FAM | | Unkn | 1b | 32.53 | 32.53 | 0,000 | 3,474E+01 | 1,541 | 3,47E+01 | 0,00E+00 | B04 | FAM | | Unkn | 1b | 31.75 | 31.75 | 0,000 | 6,158E+01 | 1,789 | 6,16E+01 | 0,00E+00 |
| Quantification Data | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Well | Floor | Target | Content | Sample | Cq | Cq Mean | Cq Std. Dev | Starting Quantity (SQ) | Log Starting Quantity | SQ Mean | SQ Std. Dev | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| A01 | FAM | | Std | HCV/IC | 18.88 | 18.88 | 0,000 | 1,000E+06 | 6,000 | 1,00E+06 | 0,00E+00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| A02 | FAM | | Std | HCV/IC | 24.28 | 24.28 | 0,000 | 1,000E+04 | 4,000 | 1,00E+04 | 0,00E+00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| A03 | FAM | | Std | HCV/IC | 28.19 | 28.19 | 0,000 | 1,000E+03 | 3,000 | 1,00E+03 | 0,00E+00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| A04 | FAM | | Std | HCV/IC | 31.95 | 31.95 | 0,000 | 5,000E+01 | 1,699 | 5,00E+01 | 0,00E+00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| A05 | FAM | | Std | HCV/IC | 34.39 | 34.39 | 0,000 | 1,000E+01 | 1,000 | 1,00E+01 | 0,00E+00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| B01 | FAM | | Unkn | 1a | 33.33 | 33.33 | 0,000 | 1,922E+01 | 1,284 | 1,92E+01 | 0,00E+00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| B02 | FAM | | Unkn | 1a | 32.61 | 32.61 | 0,000 | 3,282E+01 | 1,516 | 3,28E+01 | 0,00E+00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| B03 | FAM | | Unkn | 1b | 32.53 | 32.53 | 0,000 | 3,474E+01 | 1,541 | 3,47E+01 | 0,00E+00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| B04 | FAM | | Unkn | 1b | 31.75 | 31.75 | 0,000 | 6,158E+01 | 1,789 | 6,16E+01 | 0,00E+00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Figure 1.1: LIPSGENE[®] Pathogen detection kits: Short Protocol-at-a-glance.