

DNA
DIAGNOSTIC

Instruction For Use

HemaVision[®]-7Q

Screening test for 7 chromosome
translocations and more than 40
breakpoints associated with leukemia

Instruction For Use

Cat No. HV01-7Q
DNA Diagnostic A/S
www.dna-diagnostic.com
Revision 2025.02.25



HemaVision[®]-7Q

Multiplex RT-PCR test

Screens for 7 leukemia associated translocations

t(1;19) (q23;p13) (TCF3::PBX1)
t(4;11) (q21;q23) (KMT2A::AFF1)
t(8;21) (q22;q22) (RUNX1::RUNX1T1)
t(9;22) (q34;q11) (BCR::ABL1)
t(12;21) (p13;q22) (ETV6::RUNX1)
t(15;17) (q24;q21) (PML::RARA)
inv(16) (p13;q22) (CBFB::MYH11)

Instruction For Use for HemaVision[®]-7Q

Cat. No. HV01-7Q

12 tests per kit

Manufacturer



DNA Diagnostic A/S

Voldbjergvej 14

8240 Risskov

Denmark

Homepage: www.dna-diagnostic.com

Email: info@dna-diagnostic.com

Phone: 0045 87323050

TRADEMARKS AND LICENSES:

HemaVision is a registered trademark of DNA Diagnostic A/S.

Cy5 is a registered trademark of GE Healthcare.

Mx3005P, Stratagene and Agilent are registered trademarks of Agilent Technologies, Inc.

Black Hole Quencher® (BHQ®), CAL Fluor® and Quasar® dye technology incorporated in this product are used under licensing agreement with Biosearch Technologies, Inc. and protected by U.S. and world-wide patents issued or in application.

TABLE OF CONTENTS

| | |
|--|----|
| 1. PURPOSE OF THE TEST..... | 4 |
| 2. PRINCIPLES OF TEST..... | 4 |
| 3. KIT COMPONENTS AND STORAGE..... | 5 |
| 4. REQUIRED EQUIPMENT..... | 6 |
| 5. PRECAUTIONS..... | 6 |
| 6. PROTOCOL AT A GLANCE..... | 8 |
| 7. DETAILED PROTOCOL..... | 8 |
| Step 7.1 RNA Preparation..... | 8 |
| Step 7.2 cDNA Synthesis..... | 8 |
| Step 7.3 qPCR..... | 9 |
| Step 7.4 Analysis & Interpretation..... | 11 |
| 8. INTERPRETATION TABLE..... | 15 |
| 9. HGNC GENE NAMES AND NCBI ACCESSION NUMBERS..... | 16 |
| 10. REVISION HISTORY..... | 17 |

1. PURPOSE OF THE TEST (INTENDED USE)

HemaVision®-7Q is a 4 hour CE IVD marked in vitro diagnostic test for qualitative screening of 7 chromosome translocations associated with chronic and acute leukemia.

HemaVision®-7Q is intended for testing total RNA samples from human blood or bone marrow for presence of chromosomal translocations associated with leukemia. The test is qualitative. Each kit contains 12 tests and each test is single use only. Tests should be performed, and results should be analysed by professionals only. The test is intended for use as an adjunct to evaluation of Leukemia in conjunction with other clinicopathological factors (aid to diagnosis). HemaVision®-7Q is not intended for quantitative tests, nor Minimal Residual Disease (MRD) and follow-up tests.

HemaVision®-7Q detects RNA transcripts from fusion genes using a RT-qPCR procedure. Alternative splice variants are also detected.

HemaVision®-7Q detects the following 7 translocations:

| |
|------------------------------------|
| t(1;19) (q23;p13) (TCF3::PBX1) |
| t(4;11) (q21;q23) (KMT2A::AFF1) |
| t(8;21) (q22;q22) (RUNX1::RUNX1T1) |
| t(9;22) (q34;q11) (BCR::ABL1) |
| t(12;21) (p13;q22) (ETV6::RUNX1) |
| t(15;17) (q24;q21) (PML::RARA) |
| inv(16) (p13;q22) (CBFB::MYH11) |

2. PRINCIPLES OF THE TEST

HemaVision®-7Q is a RT-qPCR based assay for detection of leukemia associated fusion gene transcripts in total RNA from whole blood or bone marrow samples. Included in the kit are ready to use cDNA and qPCR Master Mixes. cDNA is synthesized by adding purified total RNA to the HemaVision®-7Q ready to use cDNA reaction mix. The resulting cDNA is added to 7 ready to use qPCR reaction tubes, which contain specific PCR primers and probes for detection of fusion genes, one reference gene and an internal amplification control (IAC). The qPCR is performed in a real-time qPCR instrument with optical filters for detection of FAM, ROX, and CY5 fluorescence. Amplification plots and Ct (cycle at threshold) values are used for identification of the translocation and fusion gene transcript using an easy-to-use interpretation table.

HemaVision®-7Q detects fusion gene transcripts using specific PCR primers and probes. The translocation specific primers bind to exons in the fusion gene enabling amplification of the region containing the breakpoint. The primers are designed to detect multiple clinically relevant breakpoints and splice variants (Figure 1).

The qPCR master mix probes are dual labeled with a fluorophore and a quencher molecule at each end of the oligoes. During the annealing step of the PCR the probe bind to the PCR products of the previous rounds. Signals are generated in the subsequent elongation step as the 5'->3'exonuclease activity of the Taq polymerase enzyme degrades the hybridized probe. This liberates the fluorophore from the quencher, which thereby increases the fluorescence. The fluorescence is measured at the end of the elongation step of every PCR cycle.

When the fluorescence for a translocation exceeds the threshold level before cycle 35 (Ct <35) the test is positive. The qPCR's are multiplexed by the use of FAM, ROX and CY5 labeled probes. This permits two translocation tests and the IAC to run in the same tube. The identity of a positive translocation test is easily deduced from the Interpretation Table.

As a control for the functionality of the qPCR reaction and for correct transfer of cDNA aliquots to the 7 qPCR reactions, an IAC is included in the cDNA reaction mix. A translocation positive control can be ordered from DNA Diagnostic (HemaVision Q Positive Controls, HV05-PCQ).

HemaVision®-7Q also includes primers and probes for detection of the reference gene transcripts ABL-1. Detection of the reference gene is a control for the integrity of the RNA sample and functionality of both cDNA and qPCR reactions.

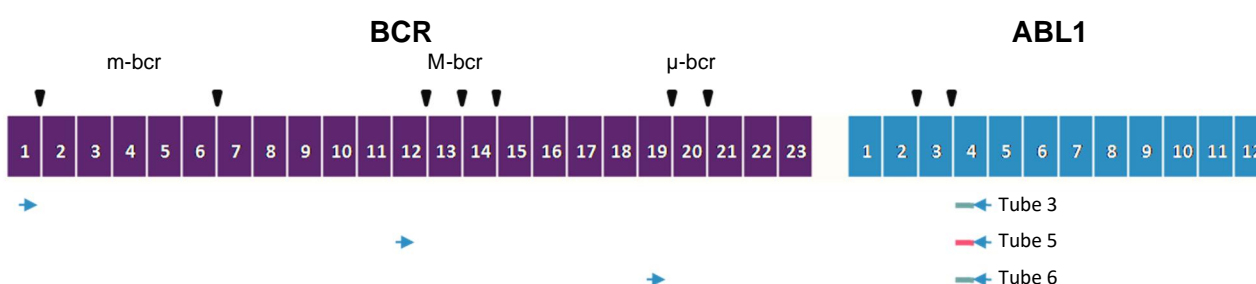


Figure 1. Primers and probes in HemaVision®-7Q are designed to detect multiple clinically relevant breakpoints. In this example primers and probe for detection of t(9;22) fusion gene transcript BCR::ABL1 are shown. Primers are depicted as blue arrows, FAM and ROX labeled probe as green and red lines and breakpoints as black triangles. Exons are numbered for the fusion genes BCR and ABL1. The three breakpoint regions (m-bcr, M-bcr, μ-bcr) of BCR are detected with the primer and probe combination of qPCR tube 3, 5, 6, respectively.

3. KIT COMPONENTS AND STORAGE

HemaVision®-7Q contains reagents for 12 tests.

Included in HemaVision®-7Q kit is the following components:

- 12 cDNA tubes with 28 µL reaction mix (0.65 mL tubes with yellow screw cap)
- 12 strips of 8 qPCR tubes containing 23 µL qPCR reaction mix.
- 12 extra strips of optical caps for the qPCR tubes.
- Two tubes with RNase free H₂O (0.65 mL tubes with blue screw cap)
- One user manual

HemaVision®-7Q is produced in 4 formats of plastic to suit various qPCR apparatus. The qPCR mix can be supplied in 0.1 mL white low profile (WLP), 0.2 mL white regular profile (WRP), 0.2 mL frosted regular profile (FRP), 0.1 mL clear Low profile (CLP) or 0.2 mL clear regular profile (CRP) PCR tubes. Adaptor plates can be supplied with the kit if the qPCR instrument requires a 96-tube plate format (e.g. Roche LightCycler 480 and some ABI models). For the Qiagen Rotor-Gene instrument, the CRP 8-tube strip can be divided in 2x4 tubes before insertion in the instrument rotor.

The cDNA reaction tubes contain reverse transcriptase, nucleotides, buffer, cDNA primers and IAC oligo template.

The qPCR tubes contain hot start Taq DNA polymerase, nucleotides, buffer and primers/probes.

Tube no. 1-6 contain primers and probes for both fusion genes and the IAC template.

Tube no. 8 contain primers and probes for a reference gene ABL1 and the IAC template.

Tube no. 7 is empty.

To confirm the orientation of the PCR strips after qPCR analysis check for FAM amplification signals in tube no. 8 and the absence of CY5 amplification signal in tube no. 7.

The kit must be stored at -20°C.

Avoid thawing and freezing of the kit.

The qPCR tubes must be protected from strong light to avoid bleaching of the probes.

4. REQUIRED EQUIPMENT

- Centrifuge for 96 well plates or PCR 8-tube strips.
- Thermal Heating Block, or Thermocycler (for cDNA synthesis)
- A qPCR instrument with filters for FAM (Abs 495 nm, Em 520 nm), ROX (Abs 585 nm, Em 605 nm) and CY5 (Abs 635 nm, Em 665 nm).
- Pipettes and sterile RNase free filtered tips.
- Gloves

5. PRECAUTIONS

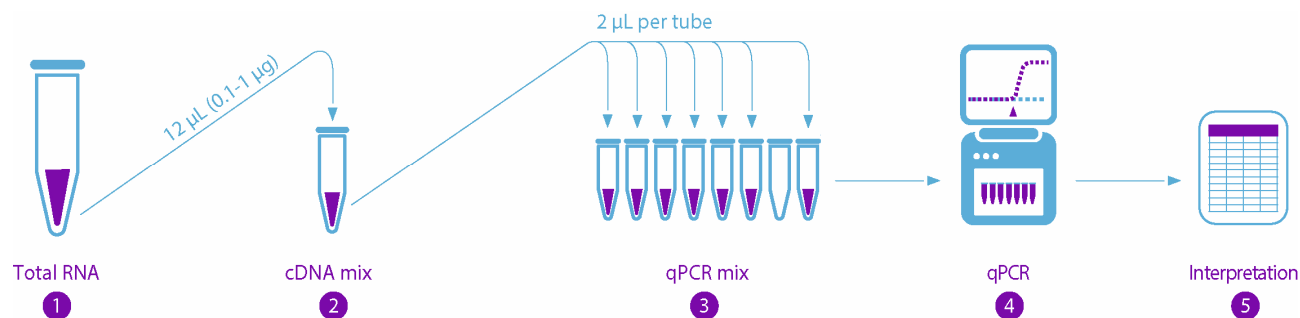
- The quality and concentration of the RNA sample greatly affects the results of this test. To minimize the risk of degradation by ribonucleases, we strongly recommend purification of total RNA immediately after blood or bone marrow extraction. Optionally, mononuclear cells can be purified prior to RNA extraction using Ficoll Hypaque. Do not freeze Ficoll purified cells without adding a denaturing solution [e.g. containing guanidinium isothiocyanate (GTC)] immediately after isolation and before freezing.
- Blood samples can be stabilized in PAXgene Blood RNA tubes, Qiagen cat. no. 762165 and bone marrow samples can be stabilized in PAXgene Bone marrow RNA tubes, Qiagen cat. no. 764114.
- Do NOT use Heparin for stabilization of blood samples.
- Always store cell samples and aqueous RNA solutions at -80°C. Even an overnight storage at -20°C may result in RNA degradation.
- When working with RNA always use gloves, as hands are a major source of ribonuclease contamination.
- The integrity and purity of RNA is important for the efficiency of the cDNA synthesis and thus detection of translocations. The quality of RNA can be checked by OD 260/280 nm measurements, agarose gel electrophoresis, or using RIN number from the Agilent Bioanalyzer or the TapeStation.

- For more guidance on specimen collection, RNA isolation and storage please refer to “ISO 20186:2019 Molecular in vitro diagnostic examinations – Specifications for pre-examination processes for venous whole blood – Part 1: isolated cellular RNA” and CLSI guidance MM13-A “Collection, Transport, Preparation and Storage of Specimens for Molecular Methods; Approved guideline.
- RT-qPCR is a very sensitive technique. Therefore, precautions must be taken to avoid false positive results caused by contamination with RNA, cDNA or PCR products from other samples.
- Dedicate four separate rooms/areas to the following activities:
 - 1) RNA extraction
 - 2) cDNA synthesis
 - 3) Addition of cDNA to qPCR master mix
 - 4) qPCR
- A set of micropipettes, aerosol barrier pipette tips, disposable gloves and clean lab coats should be kept in each of the four rooms. The work must be organized so that mixes and reaction products only moves in the direction from 1-4. NEVER move mixes or reaction products in the opposite direction.
- Laboratory workbenches, pipettes, and lab coats must be cleaned on a regular basis.
- Use of aerosol barrier pipette tips is **highly recommended** during the entire procedure. It is essential to change gloves very often when handling tubes containing RNA or cDNA. To minimize contamination avoid opening PCR tubes after amplification as they contain very high copy numbers of PCR amplicons.
- The detection of translocations can be checked with our HemaVision Q Positive Controls (HV05-PCQ)
- For more general guidance on best practice in qPCR testing please refer to the CLSI guidance document MM01: Molecular Methods for Clinical Genetics and Oncology Testing, 3rd Edition.

Safety

- Read and understand the procedure before starting.
- Normal laboratory aseptic technique should be followed at all times.
- Treat each sample as if it is infectious.
- Wear eye protection and disposable gloves during all steps of the assay.
- The products can be discarded as normal laboratory waste.
- Check the safety data sheet for the product on our website for further information or ask for a copy of it.

6. PROTOCOL AT A GLANCE



| 1: RNA denaturation | 2: cDNA synthesis | 3: Add cDNA to qPCR tubes | 4: Program qPCR Instrument | 5: qPCR analysis |
|--|--|---|--|---|
| 12 µL total RNA (0.1-1 µg) ▼ 65°C / 5 min ▼ 0°C / 1 min ▼ Spin / 1 min | Transfer to one cDNA tube ▼ Mix and spin / 1 min ▼ 42°C / 60 min ▼ 95°C / 5 min ▼ 0°C / 1 min ▼ Spin / 1 min | Spin 8-tube strip 10 seconds ▼ Remove and discard lids ▼ Add 2 µL cDNA to each of the 7 qPCR mixes ▼ Close tubes with new optical lids ▼ Spin / 1 min | 1 cycle: 95°C / 15 min ▼ 40 cycles: 95°C / 30 sec 60°C / 50 sec 72°C / 80 sec* *read FAM, ROX, CY5 | Generate amplification plots and use Interpretation Table |

Figur 2. Overview of HemaVision®-7Q protocol.

7. DETAILED PROTOCOL

7.1 RNA Preparation

Total RNA is extracted from blood or bone marrow cells. A minimum of 0.15 µg total RNA with a minimum concentration of 8 ng/µL RNA is needed for the subsequent steps.

Due to the inherent instability of RNA use patient samples as fresh as possible.

RNA can be extracted from whole blood with QIAmp RNA blood Mini Kit, Qiagen cat. no. 52304.

For blood collected in PAXgene tubes, RNA can be extracted with the PAXgene Blood RNA Kit, Qiagen cat. no. 762164.

For bone marrow collected in PAXgene tubes, RNA can be extracted with PAXgene Bone marrow RNA Kit, Qiagen cat. no. 764133.

Other total RNA extraction methods can be used, as long as RNA integrity is maintained, and inhibiting agents removed.

Total RNA should be stored at -80°C.

7.2 cDNA Synthesis

- Incubate total RNA at 65°C for 5 minutes and cool immediately on ice. Spin tube for 1 minute to collect condensate.
- Take one yellow capped tube with cDNA master mix from the HemaVision®-7Q kit stored at -20°C. Add 12 µL (0.1-1 µg) of denatured total RNA to the cDNA master mix tube (tube with yellow cap). If necessary, the RNA sample can be diluted with the provided RNase free H₂O (tube with blue cap). Mix and spin for 1 minute.
- Incubate the cDNA reaction at 42°C for 60 minutes.
Note: Incubation can be performed in the yellow capped cDNA mix tube (in thermal heating block - make sure that the tube fits the block well for efficient transfer of heat.), or by transferring mix to a 0.2mL tube and incubating 42°C for 60 minutes and 95°C for 5 minutes in a thermocycler. If transferring to another tube, a second centrifugation step of the yellow capped tube can aid in maximum recovery of sample and cDNA mix.
- Inactivate Reverse Transcriptase enzyme by heating to 95°C for 5 minutes.
- Cool the cDNA reaction tube on ice for 1 minute and spin it again for 1 minute to collect condensate.

7.3 qPCR

- For each cDNA sample use one 8-tube strip with qPCR master mix. Spin the 8-tube strip for 10 seconds to collect qPCR master mix at the bottom of tubes.
- Remove and discard the foil from 8-tube strip. Be careful to avoid cross contamination and spill of reaction mixes while removing the foil.
- Add 2 µL cDNA reaction to each of the 7 tubes containing 23 µL qPCR master mix (tube 1-6 and 8). Tube number 7 is empty and is used for orientation of the 8-tube strip, see figure 3.

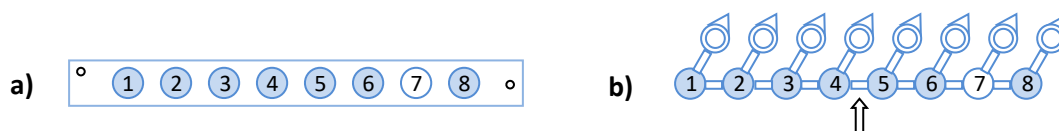


Figure 3. Orientation of qPCR tubes in 8-tube strips.

a) Positions of the 7 qPCR master mixes in the 8-tube strip formats **WLP, WRP, FRP and CLP**. Tube number 7 is empty to help orientation of the 8-tube strip. The hole in the corner and the middle of each 8-tube strip (depicted as °) marks the beginning and end respectively of the strip. The tube numbering refers to the tube numbering in the Interpretation table.

b) Positions of the 7 qPCR master mixes in the 8-tube strip format **CRP** for use in Rotor-Gene instruments. Tube number 7 is empty to help orientation of the 8-tube strip. The tube numbering refers to the tube numbering in the Interpretation table. The arrow marks the hinge to be broken before insertion into the rotor since only 4-tube strips and not 8-tube strips can be inserted in the rotor.

- Close the 8 tubes with a fresh set of optical caps supplied with the kits (or the hinged caps for CRP tubes).
- Mix contents by vortexing the tubes shortly (e.g. 5 seconds), or by flicking the tubes, and spin tubes briefly to collect liquid at the bottom.
- Insert the 8-tube strip into the qPCR instrument. The empty tube (#7) is intended for orientation of the tubes in the qPCR instrument.
- Run the qPCR reaction with the following program:

| Step | Time/Temperature | Cycles | Comment |
|------|---|--------|---|
| 1 | 15 minutes / 95°C | 1 | Activation of Taq Polymerase |
| 2 | 30 seconds / 95°C 50 seconds / 60°C 80 seconds / 72°C * | 40 | DNA amplification * Read fluorescence for FAM, ROX and CY5 at the end of each extension step |

Note: As the HemaVision®-7Q kit contains ROX labeled probes make sure that the instrument software is not set for using ROX as a passive reference dye.

7.3.1 Important notes for ABI 7500, ViiA7 and QuantStudio users

- Ramp speed: Use standard ramp speed (not fast mode).
- Targets: While defining the 3 targets FAM, ROX and CY5 choose for all targets “None” as Quencher.
- Passive reference dye: Select “None” instead of “ROX” as passive reference dye.

7.3.2 Important notes for Roche LightCycler 480 users

A Color Compensation (CC) file should be generated before the first run on the Light Cycler 480 and if the lamp has been replaced. A CC-kit with FAM, ROX and CY5 dye is available from DNA Diagnostic.

Recommended qPCR software setup:

- Melt factor: 1, Quant factor: 5, Max integration time: 2 seconds.
- Set the ramp speed to 2.2 C/sec for annealing step and 4.4 C/sec for the remaining steps.
- For the amplification step of the PCR program, under Analysis Mode choose Quantification and Acquisition Mode: Single.
- Filter choice: Either choose 4 color hydrolysis probe or manually choose FAM, Red 610 and Cy5.
- **NB. For advanced LightCycler 480 software and analysis settings please contact DNA Diagnostic for a separate technical note.**

7.3.3 Important notes for Qiagen Rotor-Gene Q users

The recommended gain setting for Rotor-Gene Q is using the Autogain function.

7.4 Analysis and Interpretation

Generate amplification plots showing:

- Thermal cycles on the X-axis.
- Background subtracted fluorescence on the Y-axis. The exact name on the Y-axis differs among qPCR instruments. See table 1.

| Instrument | Y-axis on amplification plot with background subtracted fluorescence |
|---|--|
| ABI: 7500, ViiA7, QuantStudio | ΔR_n |
| BioRad: CFX96, CFX96 Touch | RFU |
| Roche: Light Cyclers 480 | Fluorescence (using Analysis option: Abs Quant / 2nd Derivative Max for All Samples) |
| Qiagen: Rotor-Gene | Norm. Fluoro. |
| Agilent: Mx3000P / Mx3005P | dR |
| Agilent: AriaMx | ΔR |
| Bio Molecular Systems: Mic qPCR cyclers | Normalized Fluorescence |

Table 1. Examples of instrument nomenclature for the Y-axis when the amplification plot shows background subtracted fluorescence.

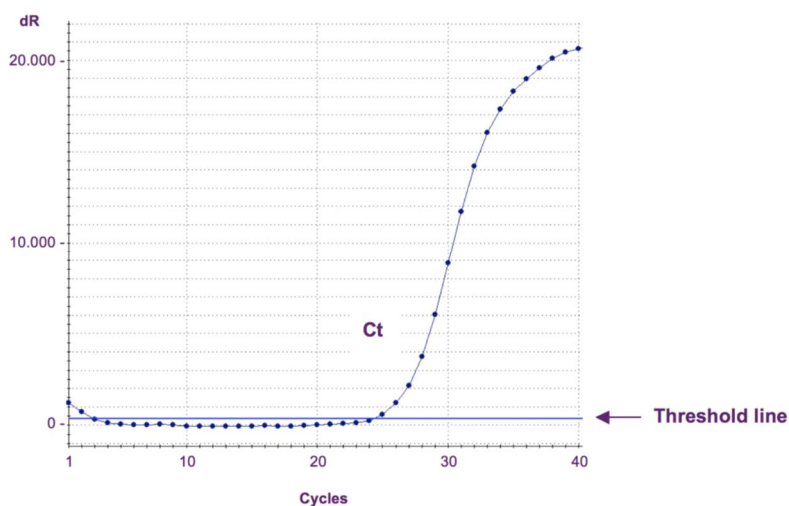


Figure 4. Amplification plot from Agilent Mx3005P with X-axis showing cycle number and Y-axis showing background subtracted fluorescence.

The threshold line is used to find the Ct value. Ct is the intersection between the amplification curve and the threshold line. The threshold line is set just above the baseline in this example resulting in a Ct value of 24.4.

Ct values are calculated automatically by the instrument. The algorithms used to calculate Ct values differ between qPCR instrument software packages. The threshold lines in the amplification plot are set manually to allow accurate Ct determination. The threshold lines are set just above the background fluorescence. The threshold lines are set sufficiently high to allow for fluctuations in background fluorescence and background fluorescence drift (a linear increase in fluorescence). The threshold level can be set manually using the following steps:

ABI; 7500, 7500 Fast, ViiA 7: In the Analysis window, choose “Plot Type: deltaRn vs Cycle”, and “Graph Type: Linear”. Unmark “Auto Threshold” and “Auto Baseline”. Then click “Show Threshold” and with the mouse move the threshold line above the baseline.

ABI; QuantStudio: In the Results screen use the menu marked with an eye; choose graph type “linear”. Unmark “Auto Threshold” and “Auto Baseline”. Then click “Show Threshold” and with the mouse move the threshold line above the baseline.

Bio-Rad CFX96 and CFX96 Touch: Click the “Settings” tab, choose “Baseline Setting” and “Baseline Subtracted Curve Fit”. Using the mouse adjust the threshold level above baseline.

Qiagen Rotor-Gene Q: Click “Analysis”, choose the fluorescence to analyze, click “Linear Scale”, “Slope Correct” and “Auto-Scale”. In the right side panel, under “CT Calculation”, click the button next to “Threshold” and mark the threshold level on the amplification plot. During analysis of the Rotor-Gene Q curves, it is recommended to try with- and without the features ‘Dynamic Tube’ and ‘Slope Correct’, to obtain optimal curves. If there are baseline fluctuations (noise) in the first cycles, use the ‘Ignore First’ function for the cycles where fluctuations occur.

Roche LightCycler480: Choose “Absolut Quantification” and “Fit Points”. Then click the tab “Noise Band” and click the “Noiseband” drop down button and choose “Noiseband (Fluoresc)”. Use the mouse to set the Noise Band.

Agilent MxPro: The automatic threshold level can be used. Choose “Adaptive Baseline Correction” and “Background-based Threshold” using cycle 6-11 and a sigma multiplier of 10.

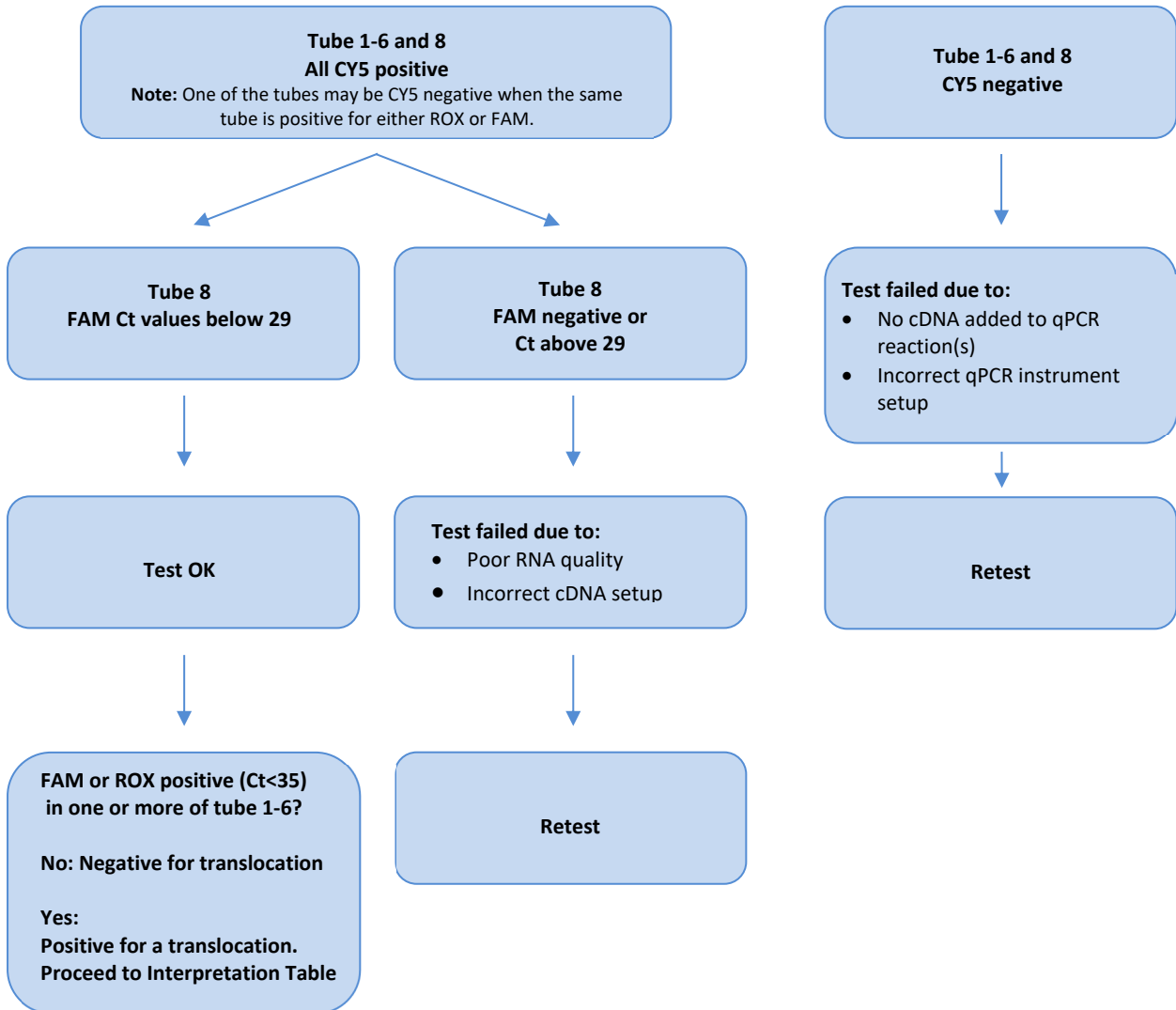
Agilent AriaMx: Use default baseline correction (adaptive) and use “Background Based Threshold” using cycle 6-11 and a sigma multiplier of 10.

Bio Molecular Systems Mic qPCR cycler: Use default analysis settings.

Note! The Ct values cannot be used for exact quantification of the fusion transcripts level since the fusion gene amplicons differ in length, resulting in different PCR efficiencies.

- Check that a CY5 signal is present from the Internal Amplification Control (IAC) in tubes no. 1-6 and 8.
The IAC CY5 fluorescence should yield Ct values between 27 and 33.
If no CY5 signals are detected the test has failed either due to no addition of cDNA to the qPCR tube(s), incorrect instrument settings or evaporation from the qPCR tube(s). Repeat the test.
Due to competition for PCR constituents CY5 Ct value above 33 may occur when the tube is also positive for FAM or ROX fluorescence.
- Check the Ct values for ABL1. The Ct values should be below: 29 in tube 8 (ABL1). Detection of the reference gene ABL1 shows RNA extraction, cDNA synthesis and qPCR have been functional.
ABL1 Ct values higher than 29 indicates the quality of the RNA may be too low to generate a valid test result. Repeat test with fresh RNA.
- Check for a “translocation” FAM or ROX signal with a Ct value below 35 in the tubes 1-6.
- Translocation tests with Ct values below 35 for FAM and ROX signals and amplification curves with exponential growth can be considered as true positive. Use the Interpretation Table to identify the specific translocation.
- Ct values above cycle 35 for FAM and ROX signals may be the result of unspecific amplification (false positive). Repeat the test to confirm or reject the result. When the second test is negative, the sample is negative for the translocation observed in the first test. When the second test is also positive, the sample may be positive for the corresponding translocation. However, we highly recommend using other diagnostic techniques to confirm the result from positive tests having Ct values above 35.

Flowchart for interpretation of data:



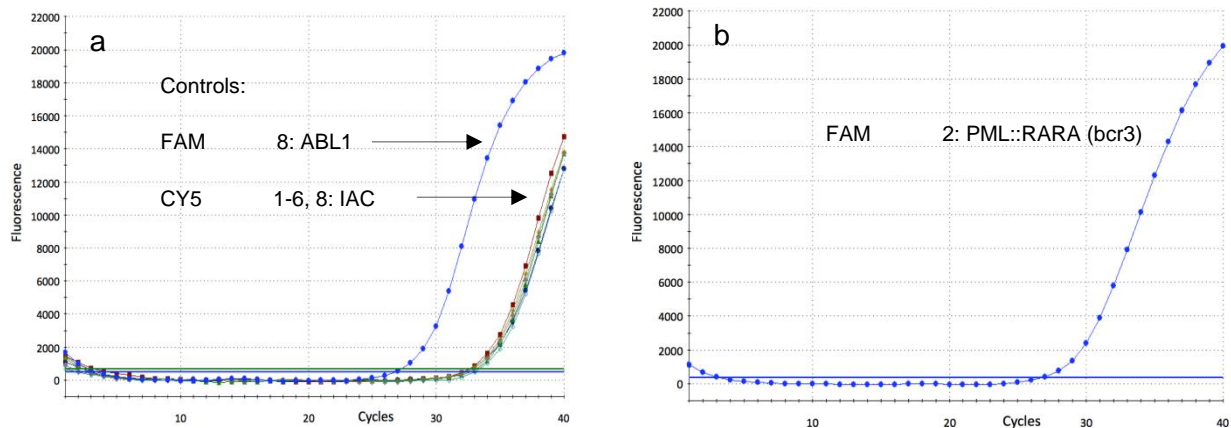


Figure 5. Example of amplification plots from a HemaVision®-7Q test.

a) The CY5 amplification plot shows all 7 IAC control curves are present and has Ct values around 32. This serves as a positive control for correct transfer of 2 μ L cDNA to all 7 qPCR tubes and functionality of the qPCR reactions.

The FAM amplification plot for the reference gene ABL1 in tubes 8 with Ct at 26. This is a positive control for both RNA quality and functionality of RT-qPCR reactions.

b) The FAM amplification plot for tube 2 shows amplification curve with Ct of 27. Therefore, the test is positive for t(15;17)(q24;q21)(PML::RARA, bcr3, S-form). See Interpretation Table.

8. INTERPRETATION TABLE

| Tube | Translocation | Fusion Gene | Fw primer - Rev primer | Fluorochrome | |
|------|-------------------|-------------------------------|------------------------|--------------|-----|
| 1 | t(1;19)(q23;p13) | TCF3::PBX1 | TCF3 ex16::PBX1 ex3 | FAM | CY5 |
| | t(12;21)(p13;q22) | ETV6::RUNX1 | ETV6 ex5::RUNX1 ex5 | ROX | CY5 |
| 2 | t(15;17)(q24;q21) | PML::RARA (bcr3, S) | PML ex3::RARA ex3 | FAM | CY5 |
| | t(4;11)(q21;q23) | KMT2A::AFF1 | KMT2A ex7::AFF1 ex9 | ROX | CY5 |
| 3 | t(9;22)(q34;q11) | BCR::ABL1 (m-bcr, P190) | BCR ex1::ABL1 ex3 | FAM | CY5 |
| | t(15;17)(q24;q21) | PML::RARA (bcr2, V) | PML ex5::RARA ex3 | ROX | CY5 |
| 4 | inv(16)(p13q22) | CBFB::MYH11 | CBFB ex4::MYH11 ex34 | FAM | CY5 |
| | t(8;21)(q22;q22) | RUNX1::RUNX1T1 | RUNX1 ex6::RUNX1T1 ex3 | ROX | CY5 |
| 5 | t(9;22)(q34;q11) | BCR::ABL1 (M-bcr, P210) | BCR ex12::ABL1 ex3 | FAM | CY5 |
| | inv(16)(p13q22) | CBFB::MYH11 | CBFB ex4::MYH11 ex31 | ROX | CY5 |
| 6 | t(9;22)(q34;q11) | BCR::ABL1 (μ -bcr, P230) | BCR ex19::ABL1 ex3 | FAM | CY5 |
| | t(15;17)(q24;q21) | PML::RARA (bcr1, L) | PML ex6a::RARA ex3 | ROX | CY5 |
| 7 | | | | | |
| 8 | ABL1 | Reference gene | ABL1 ex2::ABL1 ex3 | FAM | CY5 |

When more than one of the tubes 1-6 are positive for FAM or ROX interpret as follows:

| Positive tubes | Interpret as tube |
|-------------------------------|--|
| 2 (FAM) 3 (ROX) 6 (ROX) | 6 (ROX): t(15;17)(PML::RARA), bcr1, L-form |
| 3 (ROX) 6 (ROX) | 6 (ROX): t(15;17)(PML::RARA), bcr1, L-form |
| 2 (FAM) 6 (ROX) | 6 (ROX): t(15;17)(PML::RARA), bcr1, L-form |
| 2 (FAM) 3 (ROX) | 3 (ROX): t(15;17)(PML::RARA), bcr2, V-form |
| 3 (FAM) 5 (FAM) | 5 (FAM): t(9;22)(BCR::ABL1), M-bcr, P210 |
| 4 (FAM) 5 (ROX) | 4 (FAM): Inv(16)(CBFB::MYH11) |

Fusion genes targeted with more than one pair of amplification primers may result in positive signals in more than one tube. Use table above in combination with Interpretation Table to identify breakpoint.

9. HGNC GENE NAMES AND NCBI ACCESSION NUMBERS

| Old Abbreviation | HGNC Abbreviation | Chromosome | HGNC ID | NCBI Accession |
|------------------|-------------------|------------|------------|----------------|
| ABL | ABL1 | 9q34.1 | HGNC:76 | NG_012034.1 |
| AF4 | AFF1 | 4q21.3 | HGNC:7135 | NM_001166693.2 |
| AML1 | RUNX1 | 21q22.3 | HGNC:10471 | NG_011402.2 |
| BCR | BCR | 22q11 | HGNC:1014 | NG_009244.2 |
| CBFB β | CBFB | 16q22.1 | HGNC:1539 | NG_009281.1 |
| E2A | TCF3 | 19p13.3 | HGNC:11633 | NG_029953.2 |
| ETO | RUNX1T1 | 8q22 | HGNC:1535 | NG_023272.3 |
| MLL | KMT2A | 11q23 | HGNC:7132 | NG_027813.1 |
| MYH11 | MYH11 | 16p13.11 | HGNC:7569 | NG_009299.1 |
| PBX1 | PBX1 | 1q23.3 | HGNC:8632 | NG_028246.2 |
| PML | PML | 15q24 | HGNC:9113 | NG_029036.1 |
| RAR α | RARA | 17q21 | HGNC:9864 | NM_000964.4 |
| TEL | ETV6 | 12p13 | HGNC:3495 | NG_011443.1 |

The HUGO Gene Nomenclature Committee (HGNC) approves a unique and meaningful name for every known human gene. The table contains a list of all relevant genes for the HemaVision[®]-7Q kit, with the old gene name abbreviation and the corresponding HGNC abbreviation. Furthermore, the table contains the NCBI (www.ncbi.nlm.nih.gov/nucleotide) accession numbers for the gene or transcript sequences targeted by HemaVision[®]-7Q primers and probes.

10. REVISION HISTORY

| REVISION NUMBER | SECTION CHANGED | DATE |
|-----------------|---|------------|
| 26 | Section 1: Purpose of the test; clarification of the text. Section 5: Precautions; added more description and reference to guidances on specimen, RNA and PCR. Updated table 9 with most recent NCBI accession numbers. | 2019.07.22 |
| 27 | Added more detail to section 5: Precautions and update 7.1 RNA extraction with more guidance and detail on recommended methods. | 2019.08.26 |
| 28 | Added one more combination for PML-RARA bcr1 in table on page 15: FAM positive tube 3 and ROX positive tube 6. | 2020.05.15 |
| 29 | Correct typo in "purpose of the test" | 2021.04.19 |
| 30 | Changed from User Manual to Instruction For Use | 2021.04.23 |
| 31 | Section 4: 'Thermal Heating Block' to 'Thermal Heating Block, or Thermocycler (for cDNA synthesis)' Section 7.2: Note added to cDNA step: 'Note: Incubation can be performed in the yellow capped cDNA mix tube (in thermal heating block), or by transferring mix to a 0.2mL tube and incubating 42°C for 60 minutes and 95°C for 5 minutes in a thermocycler. If transferring to other tube, a second centrifugation step of the yellow capped tube can aid in maximum recovery of sample and cDNA mix.' Section 7.3.2: 'Ask us' changed to 'contact DNA Diagnostic' Section 7.3.3: The recommended gain setting for Rotor-Gene Q is changed from 'Green (FAM): 8, Orange (ROX): 8, Red (Cy5): 3' to 'using the Autogain function.' Section 7.4: Following added: 'During analysis of the Rotor-Gene Q curves, it is recommended to try with- and without the features 'Dynamic Tube' and 'Slope Correct', to obtain optimal curves. If there are baseline fluctuations (noise) in the first cycles, use the 'Ignore First' function for the cycles where fluctuations occur.' Section 7.4: 'QuantStudio 5' changed to 'QuantStudio' Section 7.4: 'standard analysis settings' changed 'default analysis settings' 'Trade mark' corrected to 'Trademark' | 2023.05.03 |
| 32 | Translocations 'SYMBOL-SYMBOL' replaced with 'SYMBOL::SYMBOL' according to 'HUGO Gene Nomenclature Committee (HGNC) recommendations for the designation of gene fusions' Leukemia (https://www.nature.com/articles/s41375-021-01436-6). Section 7.3: A brief mixing/vortex step of the tubes added to the protocol. The point about wearing gloves removed (redundant). Tube formats (CLP, WLP) removed (irrelevant). Section 1: Added 'HemaVision®-7Q is not intended for quantitative tests, nor Minimal Residual Disease (MRD) and follow-up tests.' Section 7.1, Added: 'Other total RNA extraction methods can be used, as long as RNA integrity is maintained, and inhibiting agents removed.' CLP tube format added. Minor changes in wording and punctuations. | 2024.04.08 |
| 33 | Section 3, page 5: 0.2 mL clear regular profile (CRP) were written twice. Corrected to 0.1 mL clear regular profile (CLP) or 0.2 mL clear regular profile (CRP). | 2024.04.10 |
| 34 | Section 7.4., IAC acceptance criteria lower limit changed from Ct 29 to Ct 27. Section 7.2: Added: '- make sure that the tube fits the block well for efficient transfer of heat' | 2024.12.19 |
| 35 | Section 3.: 0.2 mL frosted regular profile (FRP) PCR tubes added to the PCR tube formats. Text to figure 3 is also updated with FRP as tube format. | 2025.02.25 |

DNA DIAGNOSTIC

Availability / questions

Our team and distributors are always at hand to answer all your questions.

Contact us to find your nearest HemaVision® partner.

For more information, contact

DNA Diagnostic A/S

Voldbjergvej 14

DK-8240 Risskov

Denmark

Tel. +45 8732 3050

info@dna-diagnostic.com

www.dna-diagnostic.com

DNA Diagnostic A/S was established in 1992.

DNA Diagnostic A/S is an ISO 13485 certified developer, manufacturer, and worldwide supplier of PCR based CE IVD marked in vitro diagnostic kits.