

DNA
DIAGNOSTIC

Instruction For Use

HemaVision[®]-7 Positive Controls

Positive Control RNA for HemaVision[®] kits:

HemaVision[®]-7
HemaVision[®]-1;19
HemaVision[®]-4;11
HemaVision[®]-8;21
HemaVision[®]-9;22
HemaVision[®]-12;21
HemaVision[®]-15;17
HemaVision[®]-inv16

Instruction For Use

Cat No. HV05-7PC

DNA Diagnostic A/S

www.dna-diagnostic.com

Revision 2021.04.27



HemaVision[®]-7 Positive Controls

Positive Control RNA for HemaVision[®] kits:

HemaVision[®]-7

HemaVision[®]-1;19

HemaVision[®]-4;11

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HemaVision[®]-9;22

HemaVision[®]-12;21

HemaVision[®]-15;17

HemaVision[®]-inv16

Instruction For Use for HemaVision[®]-7 Positive Controls

Cat. No. HV05-7PC

One test per kit

Manufacturer 

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HemaVision®-7 Positive Controls

Contains positive control RNA for HemaVision® kits:

HemaVision®-7
HemaVision®-1;19
HemaVision®-4;11
HemaVision®-8;21
HemaVision®-9;22
HemaVision®-12;21
HemaVision®-15;17
HemaVision®-inv16

User Manual

for

HemaVision®-7PC, Cat. No. HV05-7PC

HV05-7PC contains RNA for one test of HV01-7 or one test of each kit: HV03-119, HV03-411, HV03-821, HV03-922, HV03-1221, HV03-1517, HV03-inv16

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1. PURPOSE OF THE KIT

HemaVision[®]-7PC is a CE-marked *in vitro* diagnostic kit containing 10 tubes with *in vitro* transcribed RNA diluted into wild type leucocyte RNA from the cell line HL60. The 10 *in vitro* transcribed RNA originates from transcription of 10 fusion gene constructs. These 10 tubes with of *in vitro* transcribed RNA mixed with HL60 total RNA can be used as positive controls for the 7 translocations detected by the HemaVision[®]-7 kit. Three translocations t(9;22), t(15;17), inv(16) have breakpoints separated by several hundreds of base pairs. Consequently, each of these three translocations are covered by two *in vitro* transcribed RNA. The HL60 RNA contains transcripts of the Biotinidase (BTD) gene which are detected by reaction control primers of the HemaVision[®] kits.

The HemaVision[®] single translocation kits Cat No. *HV03-119*, *HV03-411*, *HV03-821*, *HV03-922*, *HV03-1221*, *HV03-1517*, *HV03-inv16* are identical with corresponding split-out PCR primer mixes from the HemaVision[®]-7 kit. Therefore, the 10 positive control RNA mixes in HemaVision[®]-7PC can also be used to test the single translocation kits.

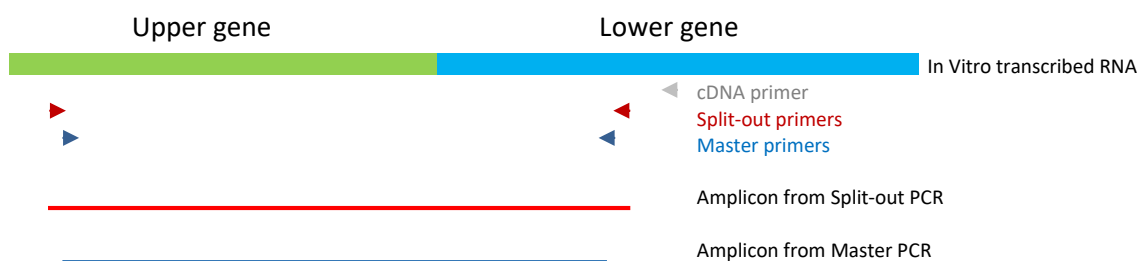


Figure 1 illustrates how HemaVision[®]-7 identifies positive control transcripts from HemaVision[®]-7PC.

Note! The HemaVision[®]-7PC kit is only for professional use.

2. PRINCIPLES OF TEST

The 10 positive control RNA's (1A-E and 2A-E) are template for synthesis of 10 cDNA's in reactions using the cDNA primer mix from HemaVision[®]-7 and Reverse Transcriptase (RT), 5xcDNA Buffer, dNTP, DTT from HemaVision[®]-RMP. The 10 cDNA's are template for five Master-1 PCR reactions (M1A-E) and five Master-2 PCR reactions (M2A-E). Each of the 10 cDNA's are also used as template for 10 Split-out PCR reaction (S1A-E and S2A-E).

PCR products are analyzed by agarose gel electrophoresis. Each PCR reaction will amplify a reaction control amplicon for the BTD gene and one translocation specific amplicon. The molecular sizes of the amplicons are shown in table 1.

Note! The 983 bp amplicon for BTD in the may not be visible in all PCR reactions due to competition for amplification with the translocation specific amplicon.

The HemaVision[®] single translocation kits Cat No. *HV03-119*, *HV03-411*, *HV03-821*, *HV03-922*, *HV03-1221*, *HV03-1517*, *HV03-inv16* can also be tested using corresponding positive control RNA from HemaVision[®]-7PC and enzymes and buffers from HemaVision[®]-PMP. The molecular sizes of the amplicons are shown in table 2.

Below examples of results, using HemaVision[®]-7PC RNA as template for testing the HemaVision[®]-7 primer mixes.

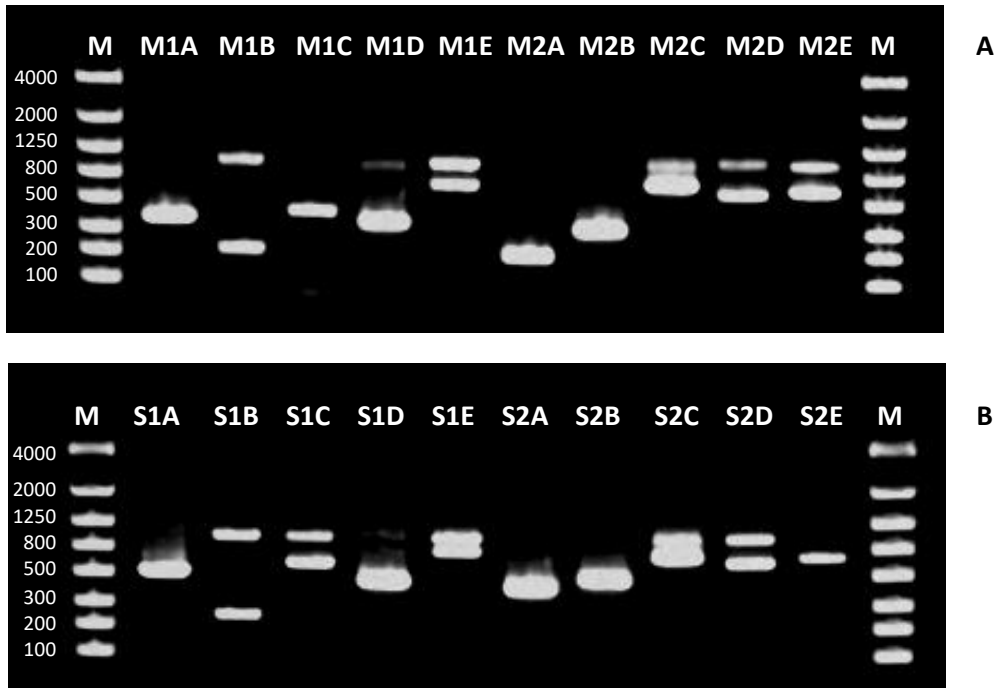


Figure 2

- A) Test of HemaVision[®]-7 cDNA primers and master primers M1 and M2 using RNA from HemaVision[®]-7PC as template. The lanes contain: M1A t(1;19), M1B t(12;21), M1C inv(16), M1D t(15;17), M1E t(9;22), M2A t(8;21), M2B t(15;17), M2C t(4;11), M2D inv(16), M2E t(9;22).
- B) Test of HemaVision[®]-7 cDNA primers and split-out primers S1A-E and S2A-E using RNA from HemaVision[®]-7PC as template. The lanes contain: S1A t(1;19), S1B t(12;21), S1C inv(16), S1D t(15;17), S1E t(9;22), S2A t(8;21), S2B t(15;17), S2C t(4;11), S2D inv(16), S2E t(9;22).

Note, the BTD reaction control amplicon of 983 bp is not visible in all lanes due to competition for amplification of the translocation specific amplicon.

cDNA Synthesis

Ten cDNA's are synthesized in 10 reactions containing the positive control RNA from HemaVision[®]-7PC, cDNA Mix (primers) from the HemaVision[®]-7 kit and Reverse Transcriptase, 5x Buffer, DTT, and dNTP from the reagent module HemaVision[®]-RMP Cat. No. HV06-RMP or HemaVision[®]-RM Cat. No. HV04-RM.

Master PCR

The ten cDNA's are used as template for 2x5 multiplex PCR amplification reactions using the HemaVision[®]-7 PCR primers M1 and M2 in reactions with HemaVision[®] DNA Polymerase, 10xbuffer, and dNTP from HemaVision[®]-RMP Cat. No. HV06-RMP (or HV04-RM and HotStarTaq DNA Polymerase 5U/μL from Qiagen). Both PCR primer mixes M1 and M2 contain five pairs of translocation specific primers and one pair of reaction control primers specific for the housekeeping gene Biotinidase (BTB). The Master PCR products are analyzed by agarose gel electrophoresis. A translocation specific band in each of the ten lanes show functionality of the cDNA and split-out PCR primers and the test has been done correctly.

Split-out PCR

The ten cDNA's are also used as template for ten multiplex PCR amplification reactions using the HemaVision®-7 split-out PCR primers S1A-E and S2A-E in reactions with HemaVision® DNA Polymerase, 10xbuffer, and dNTP from HemaVision®-RMP Cat. No. HV06-RMP (or HV04-RM and HotStarTaq DNA Polymerase 5U/μL from Qiagen).

Each Split-out PCR reaction contain only one pair of translocation specific primers and one pair of reaction control primers. The split-out PCR amplicons are analyzed by agarose gel electrophoresis. A translocation specific band in each of the ten lanes show functionality of the cDNA and split-out PCR primers and the test has been done correctly.

Interpretation of results

A RT-PCR reaction is positive when the translocation specific amplicon has the molecular size shown in Table 4.

A RT-PCR reaction is negative when the translocation specific amplicon is not present.

A RT-PCR reaction with a translocation specific amplicon having a molecular size different from the expected show the PCR primers or the control RNA was used in an incorrect order.

3. KIT COMPONENTS AND STORAGE

The HemaVision®-7PC kit Cat. No. HV05-7PC contains two boxes each with five tubes containing 10 μL in vitro transcribed RNA mixed with HL60 total RNA. The kit is shipped at -73°C and the kit must be stored at -80°C by the customer. While performing the test always keep test components on ice (0°C). Each of the ten tubes in the kit contains RNA for one cDNA reaction.

NOTE: It is essential for functionality of the HemaVision®-7PC kit also to obtain and use the reagents provided in HemaVision®-RMP kit, **Cat. No. HV06-RMP** containing: MMLV-Reverse Transcriptase; 5x cDNA buffer; DTT; dNTP, HemaVision DNA Polymerase, 10x PCR buffer or HemaVision®-RM kit, Cat. No. HV04-RM containing: MMLV-Reverse Transcriptase; 5x cDNA buffer; DTT; dNTP and HotStarTaq DNA Polymerase 5 U/μL, 10x PCR Buffer from Qiagen.

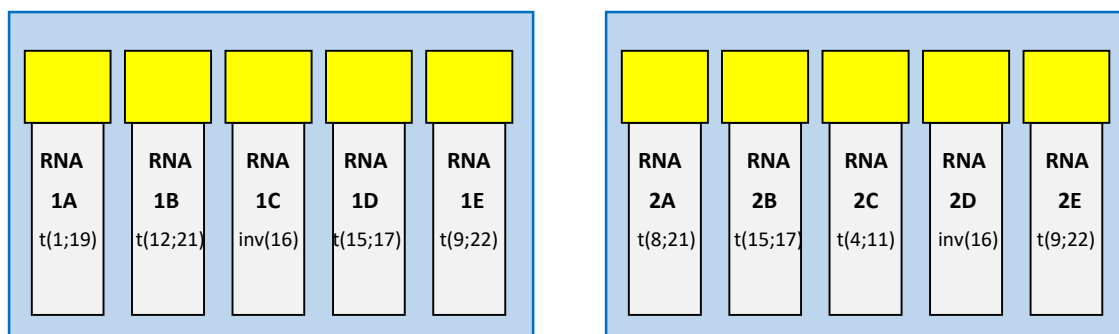


Figure 3. HemaVision®-7PC contains two boxes each with 5 tubes. Each tube contains 10 μL RNA

4. EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

Reagent Module:

HemaVision®-RMP kit Cat. No. HV06-RMP contains: MMLV-RT; 5x cDNA buffer; DTT; dNTP, HemaVision DNA Polymerase, 10x PCR buffer.

HemaVision®-RM kit Cat. No. HV04-RM contains: MMLV-RT; 5x cDNA buffer; DTT; dNTP.

HotStarTaq DNA Polymerase 5U/μL, 10x PCR buffer from Qiagen.

Master Mix room – No templates in this room:

Micropipettes, 0.5-10 µL, 20-200 µL,

HemaVision® kit Cat. No. HV06-RMP containing: MMLV-RT; 5x cDNA buffer; DTT; dNTP, HemaVision DNA Polymerase, 10x PCR buffer

Aerosol barrier micropipette tips, 0.5-10 µL, and 20-200 µL

Micro centrifuge

Ice bath

RNase free tubes

Disposable gloves

RNase free water

-20°C freezer for storage of kits (HemaVision®-7 and HemaVision®-RMP)

cDNA room:

Micropipettes, 0.5-10 µL, 20-200 µL

Aerosol barrier micropipette tips, 0.5-10 µL, and 20-200 µL

Micro centrifuge

Heating block/Water bath

Ice bath

RNase free tubes

Disposable gloves

RNase free water

-80°C freezer for storage of HemaVision®-7PC

PCR room:

Micropipettes, 0.5-10 µL, 20-200 µL

Aerosol barrier micropipette tips, 0.5-10 µL, and 20-200 µL

Micro centrifuge

Thermal Cycler

Ice bath

PCR tubes (0.1 mL or 0.2 mL) and lids

Disposable gloves

Gel electrophoresis room:

Micropipettes, 0.5-10 µL

Aerosol barrier micropipette tips, 0.5-10 µL

Micro centrifuge

Equipment for agarose gel electrophoresis

Disposable gloves

Molecular size marker (e.g. 100 bp ladder)

5. PRECAUTIONS**General precautions**

1. The quality of the RNA sample greatly affects the results of this test. To minimize the risk of degradation of RNA by ribonucleases, we strongly recommend to store RNA solutions at –80°C. When working with RNA always use gloves to avoid ribonuclease contamination from hands.
2. RT-PCR 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 to the following activities:

- 1) Master Mix production – No templates in here

- 2) cDNA synthesis
- 3) PCR
- 4) Gel electrophoresis

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 Master Mix room to cDNA room to PCR room to Gel electrophoresis room. NEVER move mixes or reaction products in the opposite direction.

3. Laboratory workbenches, pipettes, and lab coats must be cleaned on a regular basis.
4. 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 DNA. After PCR tubes must be opened with extreme care to avoid spillage of high copy number DNA products.

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.

6. PROCEDURE

Procedural notes

- Store all test components as described in section 3: *KIT COMPONENTS AND STORAGE*.
- Do not mix reagents from different lots.
- Careful pipetting technique is essential for accurate results.
- This protocol is optimized with enzymes and buffers from HemaVision[®] kit Cat. No. HV06-RMP and HemaVision[®] kit Cat. No. HV04-RM + HotStarTaq DNA Polymerase 5U/μL, 10xPCR buffer (Qiagen).
- This protocol is optimized for the ABI (Perkin Elmer) GeneAmp 9600/9700 thermal cycler. Use of another thermal cycler may require optimization by the user.

Prepare positive control RNA for use in cDNA synthesis

- 1) Transfer the HemaVision[®]-7PC RNA tubes from -80°C to ice (0°C) just before use in cDNA synthesis.
- 2) Add 10 μL RNase free H₂O to each of the positive control RNA samples.

Test of HemaVision[®]-7

Use 16 μL of the diluted positive control RNA for cDNA synthesis. Follow the instructions for cDNA synthesis and PCR from the HemaVision[®]-7 user manual. Make 10 cDNA reactions.

Test of HemaVision[®] single translocation kits Cat No. HV03-119, HV03-411, HV03-821, HV03-922, HV03-1221, HV03-1517, HV03-inv16

Use 8 μL the diluted positive control RNA for cDNA synthesis. Follow the instructions for cDNA synthesis and PCR from the HemaVision[®] single translocation kits Cat No. HV03-119, HV03-411, HV03-821, HV03-922, HV03-1221, HV03-1517, HV03-inv16.

Make one cDNA reaction for each of the kits HV03-119, HV03-411, HV03-821 and HV03-1221.

Make two cDNA reactions for each of the kits HV03-922, HV03-1517 and HV03-inv16.

Table 1: Interpretation table for test of HemaVision®-7

TEST OF HEMAVISION-7						
RNA	TRANSLOCATION	GENES	PCR primers	AMPLICON	PCR primers	AMPLICON
1A	t(1;19)(q23;p13)	TCF3(19p13.3) PBX1(PRL)(1q23.3)	M1	382 bp	S1A	530 bp
1B	t(12;21)(p13;q22)	ETV6(12p13) RUNX1(21q22.3)	M1	206 bp	S1B	241 bp
1C	inv(16)(p13;q22)	CBFB (16q22.1) MYH11 (16p13.11)	M1	417 bp	S1C	619 bp
1D	t(15;17)(q24;q21)	PML(15q24) RARA(17q21)	M1	359 bp	S1D	461 bp
1E	t(9;22)(q34;q11)	BCR(22q11) ABL1(9q34.1)	M1	696 bp	S1E	791 bp
2A	t(8;21)(q22;q22)	RUNX1(21q22.3) RUNX1T1(8q22)	M2	203 bp	S2A	413 bp
2B	t(15;17)(q24;q21)	PML(15q24) RARA(17q21)	M2	331 bp	S2B	496 bp
2C	t(4;11)(q21;q23)	KMT2A(11q23) AFF1(4q21.3)	M2	674 bp	S2C	694 bp
2D	inv(16)(p13;q22)	CBFB (16q22.1) MYH11(16p13.11)	M2	597 bp	S2D	649 bp
2E	t(9;22)(q34;q11)	BCR(22q11) ABL1(9q34)	M2	618 bp	S2E	702 bp

Table 2: Interpretation table for test of HemaVision® single translocation kits

TEST OF HEMAVISION SINGLE TRANSLOCATION KITS				
RNA	HemaVision® kit Cat. No.	TRANSLOCATION	GENES	AMPLICON
1A	HV03-119	t(1;19)(q23;p13)	TCF3(19p13.3) PBX1(PRL)(1q23.3)	530 bp
1B	HV03-1221	t(12;21)(p13;q22)	ETV6(12p13) RUNX1(21q22.3)	241 bp
1C	HV03-16	inv(16)(p13;q22)	CBFB (16q22.1) MYH11 (16p13.11)	PCR A 619 bp
1D	HV03-1517	t(15;17)(q24;q21)	PML(15q24) RARA(17q21)	PCR A 461 bp
1E	HV03-922	t(9;22)(q34;q11)	BCR(22q11) ABL1(9q34.1)	PCR A 791 bp
2A	HV03-821	t(8;21)(q22;q22)	RUNX1(21q22.3) RUNX1T1(8q22)	413 bp
2B	HV03-1517	t(15;17)(q24;q21)	PML(15q24) RARA(17q21)	PCR B 496 bp
2C	HV03-411	t(4;11)(q21;q23)	KMT2A(11q23) AFF1(4q21.3)	694 bp
2D	HV03-16	inv(16)(p13;q22)	CBFB (16q22.1) MYH11(16p13.11)	PCR B 649 bp
2E	HV03-922	t(9;22)(q34;q11)	BCR(22q11) ABL1(9q34)	PCR B 702 bp

Table 1 and 2 are used for interpretation of results observed from agarose gel electrophoresis of PCR reactions. The table lists translocations, involved genes, and molecular size of PCR amplicons.

Note: The interpretation tables has been updated according to the HUGO Gene Nomenclature Committee (HGNC) see section “7. GENE ABBREVIATIONS ACCORDING TO THE HGNC”.

Interpretation of results

- Look for a positive Biotinidase (BTD) reaction control band (983 bp) in all PCR reactions. The reaction control band can be weak or missing in the lane containing a strong translocation specific band.
- Look for a translocation specific band. Identify the translocation with Table 1 or 2. The translocation specific Split-out PCR bands have a slightly larger molecular size as the translocation specific bands in the corresponding Master PCR reactions.
- A RT-PCR reaction is positive when the translocation specific amplicon has the molecular size shown in Table 1 or 2.
- A RT-PCR reaction is negative when the translocation specific amplicon is not present.
- A RT-PCR reaction with a translocation specific amplicon different from the molecular size shown in Table 1 or 2 indicate the PCR primers or the control RNA were used in an incorrect order or the test has been contaminated with RNA or DNA.

7. GENE ABBREVIATIONS ACCORDING TO THE HGNC:








The HUGO Gene Nomenclature Committee (HGNC) approves a unique and meaningful name for every known human gene (read more at www.genenames.org). Table 3 contains a list of all relevant genes for the HemaVision®-7 kit, with the old abbreviation and the corresponding HGNC abbreviation. The chromosome position for the gene, HGNC ID number for the protein and NCBI ACCESSION number for the DNA sequence encoding the mRNA are also shown. For details go to the NCBI web site (www.ncbi.nlm.nih.gov).

Table 3

Old Abbreviation	HGNC Abbreviation	Chromosome	HGNC ID	NCBI ACCESSION
ABL	ABL1	9q34.1	76	NM_007313
AF4	AFF1	4q21.3	7135	NM_001166693
AML1	RUNX1	21q22.3	10471	NM_001754
BCR	BCR	22q11	1014	NM_004327
CBF β	CBFB	16q22.1	1539	NM_022845
E2A	TCF3	19p13.3	11633	NM_003200
ETO	RUNX1T1	8q22	1535	NM_004349
MLL	KMT2A	11q23	7132	NM_001197104
MYH11	MYH11	16p13.11	7569	NM_001040114
PBX1	PBX1	1q23.3	8632	NM_002585
PML	PML	15q24	9113	NM_033238
RAR α	RARA	17q21	9864	NM_000964
TEL	ETV6	12p13	3495	NM_001987
BTD	BTD	3p25	1122	NM_000060

REVISION HISTORY

REVISION NUMBER	SECTION CHANGED	<u>DATE</u>
7	Changed from User Manual to Instruction for Use	2021.04.26

 "Conformité Européenne" ("European Conformity")	 In vitro Diagnostic Medical Device	 Consult instructions for use
REF Catalogue Number	 Lot number	CONT Contents
 Storage temperature	 Expiry Date	 Manufacturer

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.

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*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.*