DNA
DIAGNOSTIC

User manual

HemaVision®-Q Positive Controls

Positive controls for HemaVision®-7Q and HemaVision®-28Q

USER MANUAL
Cat No. HV05-PCQ

DNA Diagnostic A/S
www.dna-diagnostic.com
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HemaVision®-Q Positive Controls

Positive controls for HemaVision®-7Q (HV01-7Q) and HemaVision®-28Q (HV01-28Q)

Translocations included are
t(15;17) (q24;q21) (PML-RARA)
inv(16) (p13;q22) (CBFB-MYH11)
t(8;21) (q22;q22) (RUNX1-RUNX1T1)
t(12;21) (p13;q22) (ETV6-RUNX1)
t(1;19) (q23;p13) (TCF3-PBX1)
t(4;11) (q21;q23) (KMT2A-AFF1)
t(9;22) (q34;q11) (BCR-ABL1)
del(1)(p32)
t(6;9) (p23;q34) (DEK-NUP214)

USER MANUAL for HemaVision Q Positive Controls
Cat. No. HV05-PCQ
Five tests per kit

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1. PURPOSE OF THE TEST

Positive controls for HemaVision® 7Q and HemaVision® 28Q is an *in vitro* diagnostic kit containing five identical tubes with in vitro transcribed RNA diluted into wild type leucocyte RNA from the cell line HL60. The *in vitro* transcribed RNA originates from transcription of 10 fusion gene constructs. These five 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®-7Q kit and 9 of the 28 translocations detected by the HemaVision®-28Q kit. The HL60 RNA contains transcripts of the three housekeeping genes, which are detected by reaction control primers and probe of the HemaVision® kits.

Note! The HemaVision® positive control kit is only for professional use.

2. PRINCIPLES OF THE TEST

The positive control RNA mix is used as template for synthesis of cDNA using the cDNA master mix from HemaVision® 7Q or HemaVision® 28Q. The cDNA is used as template for qPCR master mixes from HemaVision® 7Q or HemaVision® 28Q.

Using the positive control RNA there should be positive translocation signals in specified tubes of the HemaVision®-7Q or 28Q kit

3. KIT COMPONENTS AND STORAGE

The Positive Controls for HemaVision® 7Q and 28Q kit Cat. No. HV05-PCQ contains one box with five tubes containing 20 µL in vitro transcribed RNA mixed with HL-60 total RNA. The kit is shipped on dry ice and the kit must be stored at -80°C. While performing the test always keep test components on ice (0°C). Each of the five tubes in the kit contains RNA for one cDNA reaction.

NOTE: It is essential for functionality of kit also to obtain and use the reagents provided in HemaVision®-7Q kit, **Cat. No. HV01-7Q** or HemaVision®-28Q kit, **Cat. No. HV01-28Q**

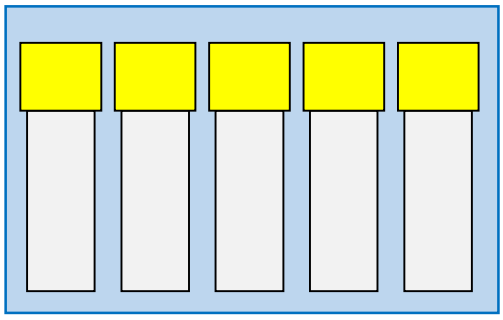


Figure 3. Positive Controls for HemaVision® 7Q and 28Q kit Cat. No. HV05-PCQ contains one box with five tubes. Each tube contains 20 µL RNA

4. REQUIRED EQUIPMENT AND MATERIALS

HemaVision® 7Q or HemaVision® 28Q kit:
HemaVision®-7Q kit Cat. no. HV01-7Q contains: cDNA master mix and qPCR master mix
HemaVision®-28Q kit Cat. no. HV01-28Q contains: cDNA master mix and qPCR master mix

Centrifuge for 96 well plates

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).

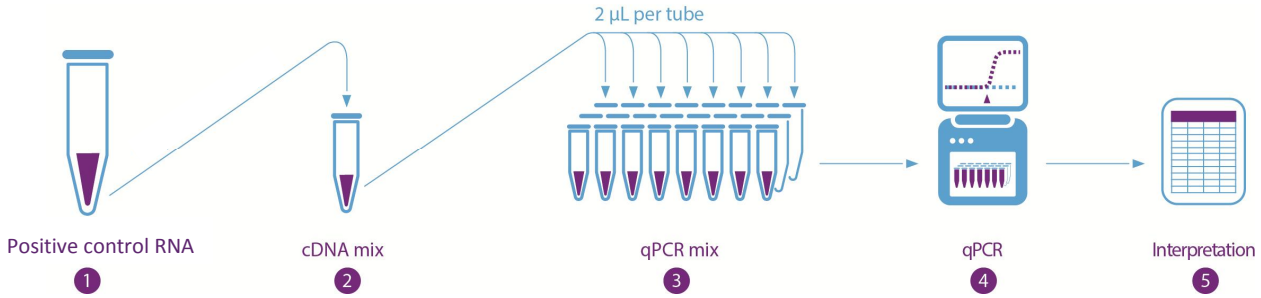
Pipettes and sterile RNase free filtered tips.

Gloves

5. PRECAUTIONS

- Always store 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 high sensitivity of DNA amplification techniques makes the reactions susceptible to DNA contamination from previous amplification reactions, potentially resulting in false positive results. To minimize the risk of contamination avoid opening qPCR tubes after amplification.
- Laboratory workbenches and pipettes must be cleaned with bleach on a regularly scheduled basis.
- Use aerosol barrier pipette tips.

6. PROTOCOL AT A GLANCE



1: RNA denaturation	2: cDNA synthesis	3: Add cDNA to qPCR tubes	4: Program qPCR Instrument	5: qPCR analysis
18 or 12 uL Positive control RNA ▼ 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 3x8-tube block or 1x8 tube block 10 seconds ▼ Remove and discard lids ▼ Add 2 uL cDNA to each of the 23 or 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

Figure 2. Overview of HemaVision®-7Q and 28Q protocol using the positive control RNA

7. DETAILED PROTOCOL

7.1 RNA Preparation, cDNA synthesis and qPCR

Positive control RNA is provided with this kit. The RNA concentration in the tubes is approx. 0.05 µg/µl. For a cDNA reaction with HemaVision® 7Q use 12 µl of positive control RNA and for a cDNA reaction with HemaVision® 28Q use 18 µl of positive control RNA. Follow the instructions in the manual for HemaVision® 7Q og HemaVision® 28Q for cDNA synthesis and qPCR.

7.2 Analysis and Interpretation

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. 1-23.

  The IAC CY5 fluorescence should yield Ct values between 29 and 34. This is a control for functionality of the qPCR reaction.

  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 34 may occur when the tube is also positive for FAM or ROX fluorescence.

- For **HemaVision® 7Q** do the following: Check the Ct value for ABL1. It should be below 30 in tube 8 (ABL1). Detection of the reference gene ABL1 shows RNA integrity and that cDNA synthesis and qPCR have been functional.

- For **HemaVision® 28Q** do the following: Check that there are Ct values below 30 for GUS, B2M and ABL1. This is a control for RNA integrity and that cDNA synthesis have been functional.

- For **HemaVision® 7Q** do the following: Check for a "translocation" FAM or ROX signal with a Ct value below 33 in the tubes 1-6. See expected positive signals in the interpretation table below. Ct values can vary with brand of qPCR machine and from run to run eg. due to variability in cDNA synthesis efficiency.

- For **HemaVision® 28Q** do the following: Check for a FAM or ROX signal with a Ct value below 33 in the "translocation" tubes 1-6, 9-15, 17-23. See expected positive signals in the interpretation table below. Ct values can vary with brand of qPCR machine and from run to run eg. due to variability in cDNA synthesis efficiency.

INTERPRETATION TABLES. Expected positive signals in HemaVision® 7Q and 28Q when using the positive control RNA can be seen in the two tables below.

HemaVision® 7Q – expected positive signals with positive control RNA

Tube	Translocation	Fusion Gene	Flourochrome	Positive signal expected
1	t(1;19)(q23;p13)	TCF3-PBX1	FAM	√
	t(12;21)(p13;q22)	ETV6-RUNX1	ROX	√
2	t(15;17)(q24;q21)	PML-RARA (bcr3, S)	FAM	√
	t(4;11)(q21;q23)	KMT2A-AFF1	ROX	√
3	t(9;22)(q34;q11)	BCR-ABL1 (m-bcr, P190)	FAM	√
	t(15;17)(q24;q21)	PML-RARA (bcr2, V)	ROX	√
4	inv(16)(p13q22)	CBFB-MYH11	FAM	-
	t(8;21)(q22;q22)	RUNX1-RUNX1T1	ROX	√
5	t(9;22)(q34;q11)	BCR-ABL1 (M-bcr, P210)	FAM	√
	inv(16)(p13q22)	CBFB-MYH11	ROX	√
6	t(9;22)(q34;q11)	BCR-ABL1 (µ-bcr, P230)	FAM	√
	t(15;17)(q24;q21)	PML-RARA (bcr1, L)	ROX	√
7				
8	ABL1	Reference gene	FAM	√

HemaVision® 28Q – expected positive signals with positive control RNA

Tube	Translocation	Gene	Flourochrome	Positive signal expected
1	t(15;17)(q24;q21)	PML-RARA (bcr2, V)	FAM	√
	inv(16)(p13;q22)	CBFB-MYH11	ROX	√
2	inv(16)(p13;q22)	CBFB-MYH11	FAM	-
	t(8;21)(q22;q22)	RUNX1-RUNX1T1	ROX	√
3	t(15;17)(q24;q21)	PML-RARA (bcr1, L)	FAM	√
	t(9;11)(p22;q23)	KMT2A-MLLT3	ROX	
4	t(15;17)(q24;q21)	PML-RARA (bcr3, S)	FAM	√
	t(9;11)(p22;q23)	KMT2A-MLLT3	ROX	-
5	t(11;19)(q23;p13.1)	KMT2A-ELL	FAM	-
	t(16;21)(p11;q22)	FUS-ERG	ROX	-
6	t(12;22)(p13;q11-12)	ETV6-MN1	FAM	-
	t(6;9)(p23;q34)	DEK-NUP214	ROX	√
7	Reference gene	GUS	FAM	√
8	Reference gene	B2M	FAM	√
9	t(1;11)(p32;q23)	KMT2A-EPS15	FAM	-
	t(6;11)(q27;q23)	KMT2A-AFDN	ROX	-
10	t(1;19)(q23;p13)	TCF3-PBX1	FAM	√
	t(12;21)(p13;q22)	ETV6-RUNX1	ROX	√
11	t(11;19)(q23;p13.3)	KMT2A-MLLT1	FAM	-
	t(4;11)(q21;q23)	KMT2A-AFF1	ROX	√
12	t(17;19)(q22;p13)	TCF3-HLF	FAM	-
	del(1)(p32)	STIL-TAL1	ROX	√
13	t(9;22)(q34;q11)	BCR-ABL1 (m-bcr, P190)	FAM	√
	t(9;9)(q34;q34)	SET-NUP214	ROX	-
14	t(11;19)(q23;p13.3)	KMT2A-MLLT1	FAM	-
	t(9;22)(q34;q11)	BCR-ABL1 (M-bcr, P210)	ROX	√
15	t(9;22)(q34;q11)	BCR-ABL1 (µ-bcr, P230)	FAM	√
	t(11;17)(q23;q21)	ZBTB16-RARA	ROX	-
16	Reference gene	ABL1	FAM	√
17	t(9;12)(q34;p13)	ETV6-ABL1	FAM	-
	t(5;12)(q33;p13)	ETV6-PDGFRB	ROX	-
18	t(10;11)(p12;q23)	KMT2A-MLLT10	FAM	-
	t(1;11)(q21;q23)	KMT2A-MLLT11	ROX	-
19	t(X;11)(q13;q23)	KMT2A-FOXO4	FAM	-
	t(11;17)(q23;q21)	KMT2A-MLLT6	ROX	-
20	t(3;21)(q26;q22)	RUNX1-MECOM	FAM	-
	t(10;11)(p12;q23)	KMT2A-MLLT10	ROX	-
21	t(5;17)(q35;q21)	NPM1-RARA	FAM	-
	t(3;5)(q25.1;q35)	NPM1-MLF1	ROX	-
22	t(10;11)(p12;q23)	KMT2A-MLLT10	FAM	-
	t(3;21)(q26;q22)	RUNX1-MECOM	ROX	-
23	t(10;11)(p12;q23)	KMT2A-MLLT10	ROX	-
24	-	-	-	-

- Translocation tests with Ct values below 33 for FAM and ROX signals and amplification curves with exponential growth and an S-shaped amplification curve can be considered as true positive. Use the Interpretation Table to identify the specific translocation.

- Ct values above cycle 33 for FAM and ROX signals may arise as a result of unspecific amplification (false positive). This can happen using the positive control RNA – it is a result of the complex mixture of templates contained in this sample.

8. HGNC GENE NAMES AND NCBI ACCESSION NUMBERS

The HUGO Gene Nomenclature Committee (HGNC) approves a unique and meaningful name for every known human gene (read more at www.genenames.org). The table below contains a list of all relevant genes for the positive control 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 of gene abbreviations

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_001166693.1
AML1	RUNX1	21q22.3	HGNC:10471	NG_011402.2
BCR	BCR	22q11	HGNC:1014	NG_009244.1
CAN	NUP214	9q34	HGNC:8064	NG_023371.1
CBFβ	CBFB	16q22.1	HGNC:1539	NG_009281.1
DEK	DEK	6p23	HGNC:2768	NM_003472.3
E2A	TCF3	19p13.3	HGNC:11633	NG_029953.1
ETO	RUNX1T1	8q22	HGNC:1535	NG_023272.2
MLL	KMT2A	11q23	HGNC:7132	NG_027813.1
MYH11	MYH11	16p13.11	HGNC:7569	NG_009299.1
PBX1	PBX1	1q23.3	HGNC:8632	NG_028246.1
PML	PML	15q24	HGNC:9113	NG_029036.1
RARα	RARA	17q21	HGNC:9864	NM_000964.3
SIL1	STIL	1p32	HGNC:10879	NG_012126.1
Tal1	TAL1	1p32	HGNC:11556	NM_003189.2
TEL	ETV6	12p13	HGNC:3495	NG_011443.1

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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.