

Leukemia Translocation Panel V2

Real-Time PCR assay

- Detects AML1-ETO, CBFB-MYH11, MLL-AF4, TEL-AML1, E2A-PBX1, and PML-RARA fusion transcripts
- One-step cDNA synthesis
- 24 tests per kit
- Available as RUO (US) / IVD (EU)

INTENDED USE:

The Leukemia Translocation Panel is a one-step detection kit intended for the relative quantitative detection of the following fusion transcripts in bone marrow or peripheral blood samples. This kit is intended for research use only. For research use only in the U.S. Not for use in diagnostic procedures. For in vitro diagnostic use in the European Union.

Disease	Translocations	Variants
AML	t(8;21)	AML1/ETO (e5/e2)
	Inv(16)	CBFB/MYH11 Type A (e5/e12) CBFB/MYH11 Type A (with SNP) CBFB/MYH11 Type D (e5/e8) CBFB/MYH11 Type E (e5/e7)
APL	t(15;17)	PML/RARα (bcr1) PML/RARα (bcr2) PML/RARα (bcr3)
ALL	t(4;11)	MLL/AF4 (e9/e4) MLL/AF4 (e9/e4, 5bp ins) MLL/AF4 (e9/e5) MLL/AF4 (e10/e4) MLL/AF4 (e10/e5) MLL/AF4 (e10/e4, 5bp ins) MLL/AF4 (e10/e5) MLL/AF4 (e11/e4) MLL/AF4 (e11/e5)
		TEL/AML1 (e5/e2)
		E2A/PBX1 (e13/e2)
	t(12;21)	
	t(1;19)	

PACKAGE CONTENTS

This product contains the following materials sufficient for 24 reactions, including samples and controls.

Description	Tubes
One-Step No ROX Reaction Mix (2X)	1
One-Step No ROX Enzyme (20X)	1
V2 Primer Mix 1	1
V2 Primer Mix 2	1
Molecular Grade Water	1
Positive Control Mix For LEUKMP-RT24	1

HANDLING AND STORAGE

This product is shipped on frozen ice packs. The contents of the shipment should be stored at -25°C to -15°C in a non-frost-free freezer immediately upon receipt.

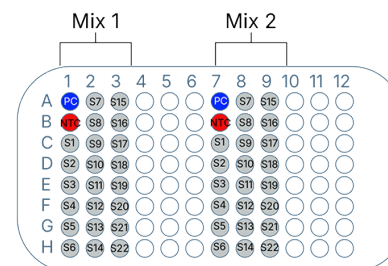
- Store all unopened components in original containers.
- Primer/probe mixes should be protected from light at all times to prevent photobleaching of the fluorescent dyes.
- Centrifuge the tubes before opening.
- This product will maintain its performance through the expiration date printed on the box label. Its performance is not guaranteed after the expiration date.

FOR USE WITH:

- Bio-Rad CFX96
- Applied Biosystems® - QuantStudio™ 5

PLATE SETUP

A single 96-well PCR plate can accommodate a single experiment analyzing 22 unknown samples, PC (P) and NTC (N).



PRINCIPLE OF THE ASSAY

LEUKMP is a one-step reverse transcription and qPCR assay designed to quantitatively detect 6 fusion genes (i.e., AML1-ETO, CBFB-MYH11, MLL-AF4, TEL-AML1, E2A-PBX1, and PML-RARA) commonly found in childhood and adult leukemias. This kit has been formulated for highly reproducible first-strand cDNA synthesis and subsequent real-time PCR in a single tube. The LEUKMP assay can be used on RNA extracted from blood or bone marrow samples. The assay includes two qPCR reactions, each of which detect three target fusions using gene-specific primers and probes (labeled by FAM, CY5 and ROX) and an internal control (ABL; labeled by CFO560/VIC/HEX).

Reporter	Reaction 1	Reaction 2
	Acute myeloid leukemia (AML)	Acute lymphocytic leukemia (ALL)
FAM	AML1-ETO t(8;21)(q22;q22)	MLL-AF4 t(4;11)(q21;q23)
CY5	CBFB-MYH11 Inv(16)(p13;q22)	TEL-AML1 t(12;21)(p13;q22)
ROX	PML-RARA t(15;17)(q24;q21)	E2A-PBX1 t(1;19)(q23;p13)
CFO560/VIC/HEX	ABL (Reference gene)	ABL (Reference gene)

The LEUKMP assay utilizes hydrolysis probes to detect leukemia-associated fusion genes and ABL amplicons (reference gene). The use of positive control (PC) enables the relative quantification of a sample's target-to-reference gene ratio in respect to the quantitative PC. The comparative Ct method (Pfaffl method) allows the determination of fusion gene ratios without running individual standards of known copy number.

ASSAY PERFORMANCE

Limit of Blank

Limit of blank was assessed on QuantStudio™5 and CFX96 real-time PCR instruments, using over 100 measurements of WT total RNA from lymphocytes and leukemia cell lines and bone marrow samples, tested at various inputs within the assay's internal control Ct range. No FAM, CY5 or ROX signals were detected under the assay's cutoff in any of the tested samples. No signal was detected when testing molecular grade water (NTC).

LoB Results on QuantStudio™ 5

Reaction	Sample	N	Average				SD			
			VIC	FAM	CYS	ROX	VIC	FAM	CYS	ROX
Reaction 1	Bone Marrow 140 ng	8	24.60	45.00	45.00	45.00	0.06	0.00	0.00	0.00
	HL-60 25ng	9	25.35	45.00	45.00	45.00	0.10	0.00	0.00	0.00
	HL-60 50ng	8	25.35	45.00	45.00	45.00	0.06	0.00	0.00	0.00
	HL-60 250ng	8	22.04	45.00	45.00	45.00	0.08	0.00	0.00	0.00
	HL-60 500ng	8	21.65	45.00	45.00	45.00	0.09	0.00	0.00	0.00
	Lymphocytes 25ng	32	25.75	45.00	45.00	45.00	0.86	0.00	0.00	0.00
	Lymphocytes 50ng	4	25.50	45.00	45.00	45.00	0.09	0.00	0.00	0.00
	Lymphocytes 250ng	36	22.61	45.00	44.95	45.00	0.67	0.00	0.31	0.00
Reaction 2	Bone Marrow 140 ng	8	24.54	45.00	45.00	45.00	0.12	0.00	0.00	0.00
	HL-60 25ng	9	25.40	45.00	45.00	45.00	0.15	0.00	0.00	0.00
	HL-60 50ng	10	25.60	45.00	45.00	45.00	0.14	0.00	0.00	0.00
	HL-60 250ng	8	22.07	45.00	45.00	45.00	0.07	0.00	0.00	0.00
	HL-60 500ng	10	22.07	45.00	45.00	45.00	0.06	0.00	0.00	0.00
	Lymphocytes 25ng	31	25.67	45.00	45.00	45.00	0.82	0.00	0.00	0.00
	Lymphocytes 50ng	11	25.53	45.00	45.00	45.00	0.20	0.00	0.00	0.00
	Lymphocytes 250ng	32	22.51	45.00	45.00	45.00	0.59	0.00	0.00	0.00

LoB Results on CFX96

Reaction	Sample	N	Average				SD			
			VIC	FAM	CYS	ROX	VIC	FAM	CYS	ROX
Reaction 1	Bone Marrow 250 ng	4	25.14	45.00	45.00	45.00	0.03	0.00	0.00	0.00
	HL-60 25ng	4	26.16	45.00	45.00	45.00	0.03	0.00	0.00	0.00
	HL-60 50ng	8	25.35	45.00	45.00	45.00	0.04	0.00	0.00	0.00
	HL-60 250ng	7	23.17	45.00	45.00	45.00	0.44	0.00	0.00	0.00
	HL-60 500ng	8	22.23	45.00	45.00	45.00	0.02	0.00	0.00	0.00
	Lymphocytes 25ng	24	26.49	45.00	45.00	45.00	0.27	0.00	0.00	0.00
	Lymphocytes 250ng	24	23.25	45.00	45.00	45.00	0.21	0.00	0.00	0.00
	Lymphocytes 500ng	24	23.25	45.00	45.00	45.00	0.21	0.00	0.00	0.00
Reaction 2	Bone Marrow 250 ng	4	24.71	45.00	45.00	45.00	0.26	0.00	0.00	0.00
	HL-60 25ng	4	26.08	45.00	45.00	45.00	0.04	0.00	0.00	0.00
	HL-60 50ng	10	25.74	45.00	45.00	45.00	0.04	0.00	0.00	0.00
	HL-60 250ng	7	22.86	45.00	45.00	45.00	0.22	0.00	0.00	0.00
	HL-60 500ng	10	22.30	45.00	45.00	45.00	0.04	0.00	0.00	0.00
	Lymphocytes 25ng	20	26.52	45.00	45.00	45.00	0.30	0.00	0.00	0.00
	Lymphocytes 250ng	20	25.79	45.00	45.00	45.00	0.14	0.00	0.00	0.00
	Lymphocytes 500ng	20	23.18	44.96	45.00	45.00	0.28	0.17	0.00	0.00

Limit of Detection at the Minimum Input

Limit of detection at the minimum input was assessed using synthetic DNA fusion controls (gBlocks) diluted to 1-8 copies/μl in the background of 5 ng/μl lymphocyte cell-line RNA. Each sample was tested by loading 5 μl per well (that is 5-40 copies in 25 ng RNA per reaction). 32 or more replicates were tested per target fusion on QuantStudio™ 5 and CFX96 Real-Time PCR instruments.

LoD at the Minimum Input on QuantStudio™ 5

Reaction	Fusion	Copies per Reaction	N	Correct Call	Ref (VIC) Average Ct	Ref SD	Target Average Ct	Target SD
Reaction 1	AML-ETO e5e2	40	32	100%	25.94	0.44	31.00	0.68
	PML-RARA bcr1	32	32	100%	25.90	0.46	35.90	0.67
	PML-RARA bcr2	5	32	97%	25.99	0.45	36.45	1.24
	PML-RARA bcr3	5	32	97%	25.96	0.44	36.71	1.19
	CBFB-MYH11 Type A (e5e12)	5	32	97%	25.90	0.46	36.04	2.09
	CBFB-MYH11 Type A (with snp)	10	32	100%	25.80	0.47	34.36	0.71
	CBFB-MYH11 Type D (e5e8)	10	40	100%	25.58	0.29	33.58	0.96
	CBFB-MYH11 Type E (e5e7)	10	32	97%	25.82	0.51	36.20	1.09
Reaction 2	E2A-PBX1 (e13e2)	5	36	97%	25.84	0.42	35.01	1.99
	TEL-AML1 (e5e2)	5	36	100%	25.74	0.48	33.28	1.59
	MLL-AF4 e9e4 (5bp ins)	5	36	97%	25.81	0.48	33.58	0.92
	MLL-AF4 e9e4	5	36	97%	25.72	0.47	36.15	1.87
	MLL-AF4 e9e5	5	36	100%	25.75	0.43	34.80	0.79
	MLL-AF4 e10e4 (5bp ins)	5	36	100%	25.82	0.45	35.13	0.87
	MLL-AF4 e10e4	10	36	100%	25.69	0.42	34.03	0.58
	MLL-AF4 e10e5	10	36	100%	25.64	0.42	33.98	0.79
	MLL-AF4 e11e4	40	40	100%	25.70	0.23	33.20	0.70
	MLL-AF4 e11e5	40	36	97%	25.69	0.42	34.44	1.95

LoD at the Minimum Input on CFX96

Reaction	Fusion	Copies per Reaction	N	Correct Call	Ref (HEX) Average Ct	Ref SD	Target Average Ct	Target SD
Reaction 1	AML-ETO e5e2	40	40	100%	25.60	0.28	32.46	0.64
	PML-RARA bcr1	10	40	100%	26.57	0.27	36.29	0.74
	PML-RARA bcr2	5	40	98%	26.57	0.28	37.10	1.51
	PML-RARA bcr3	10	44	100%	26.63	0.27	36.07	0.68
	CBFB-MYH11 Type A (e5e12)	5	40	95%	26.57	0.24	36.94	1.34
	CBFB-MYH11 Type A (with snp)	10	40	100%	26.60	0.27	35.79	0.52
	CBFB-MYH11 Type D (e5e8)	10	40	100%	26.59	0.32	35.70	0.68
	CBFB-MYH11 Type E (e5e7)	10	40	100%	26.55	0.28	36.40	0.54
Reaction 2	E2A-PBX1 (e13e2)	10	40	100%	26.59	0.25	34.35	0.46
	TEL-AML1 (e5e2)	20	40	100%	26.57	0.25	33.86	0.33
	MLL-AF4 e9e4 (5bp ins)	5	40	98%	26.58	0.26	35.33	1.74
	MLL-AF4 e9e4	5	40	95%	26.59	0.25	35.61	1.84
	MLL-AF4 e9e5	5	40	100%	26.60	0.24	35.14	0.84
	MLL-AF4 e10e4 (5bp ins)	5	40	100%	26.55	0.27	35.12	0.95
	MLL-AF4 e10e4	10	40	100%	26.63	0.28	34.05	0.51
	MLL-AF4 e10e5	10	40	100%	26.57	0.23	34.10	0.66
	MLL-AF4 e11e4	20	44	98%	26.66	0.28	34.22	0.54
	MLL-AF4 e11e5	20	40	100%	26.56	0.25	33.83	0.61

Limit of Detection at the Maximum Input

Limit of detection at the maximum input was assessed using synthetic DNA fusion controls (gBlocks) at 5-10 copies/μl (25-50 copies per reaction) in the background of 50 ng/μl lymphocyte cell-line RNA (250 ng per reaction). Samples were tested on a QuantStudio™ 5 and CFX96 Real-Time PCR instruments.

LoD at the Maximum Input on QuantStudio™ 5

LoD at the maximum input was tested at 25-50 total copies of the target fusion in 250 ng RNA per reaction. Each target was tested with 20 replicates on a QuantStudio™ 5.

Reaction	Fusion	Copies per Reaction	N	Correct Call	Ref (VIC) Average Ct	Ref SD	Target Average Ct	Target SD
Reaction 1	AML-ETO e5e2	50	20	100%	22.57	0.63	31.59	0.83
	PML-RARA bcr1	25	20	100%	22.56	0.74	34.10	0.66
	PML-RARA bcr2	25	20	100%	22.64	0.65	33.36	0.44
	PML-RARA bcr3	25	20	100%	22.62	0.65	33.77	0.67
	CBFB-MYH11 Type A (e5e12)	25	20	100%	22.56	0.63	32.93	0.78
	CBFB-MYH11 Type A (with snp)	25	20	100%	22.48	0.65	32.91	0.69
	CBFB-MYH11 Type D (e5e8)	25	20	100%	21.97	0.13	32.45	0.32
	CBFB-MYH11 Type E (e5e7)	25	20	100%	22.51	0.65	34.26	0.92
Reaction 2	E2A-PBX1 (e13e2)	25	20	100%	22.52	0.63	32.24	0.44
	TEL-AML1 (e5e2)	25	20	100%	22.47	0.68	32.31	0.56
	MLL-AF4 e9e4 (5bp ins)	25	20	100%	22.55	0.63	32.62	0.66
	MLL-AF4 e9e4	25	20	100%	22.47	0.69	32.84	0.55
	MLL-AF4 e9e5	25	20	100%	22.47	0.69	32.65	0.51
	MLL-AF4 e10e4 (5bp ins)	25	20	100%	22.51	0.65	32.61	0.69
	MLL-AF4 e10e4	25	20	100%	22.45	0.69	32.78	0.63
	MLL-AF4 e10e5	25	20	100%	22.37	0.68	32.60	0.65
	MLL-AF4 e11e4	25	20	95%	22.40	0.71	35.91	1.58
	MLL-AF4 e11e5	25	20	95%	22.42	0.67	33.97	0.94

LoD at the Maximum Input on CFX96

LoD at the maximum input was tested at 25-50 total copies of the target fusion in 250 ng RNA per reaction. Each target was tested with 16 replicates on a CFX96.

Reaction	Fusion	Copies per Reaction	N	Correct Call	Ref (HEX) Average Ct	Ref SD	Target Average Ct	Target SD
Reaction 1	AML-ETO e5e2	50	16	100%	23.35	0.04	31.76	0.25
	PML-RARA bcr1	25	16	100%	23.31	0.05	34.64	0.30
	PML-RARA bcr2	25	16	100%	23.32	0.10	34.19	0.32
	PML-RARA bcr3	25	16	100%	23.36	0.08	34.89	0.42
	CBFB-MYH11 Type A (e5e12)	25	16	100%	23.44	0.25	34.56	0.52
	CBFB-MYH11 Type A (with snp)	25	16	100%	23.40	0.09	34.27	0.33
	CBFB-MYH11 Type D (e5e8)	25	16	100%	23.46	0.05	34.25	0.21
	CBFB-MYH11 Type E (e5e7)	25	16	100%	23.39	0.10	35.00	0.31
Reaction 2	E2A-PBX1 (e13e2)	25	16	100%	23.28	0.13	32.88	0.33
	TEL-AML1 (e5e2)	25	16	100%	23.31	0.06	33.45	0.31
	MLL-AF4 e9e4 (5bp ins)	25	16	100%	23.31	0.05	32.46	0.30
	MLL-AF4 e9e4	25	16	100%	23.33	0.06	32.67	0.41
	MLL-AF4 e9e5	25	16	100%	23.37	0.07	32.59	0.32
	MLL-AF4 e10e4 (5bp ins)	25	16	100%	23.31	0.10	32.43	0.24
	MLL-AF4 e10e4	25	16	100%	23.36	0.10	32.78	0.42
	MLL-AF4 e10e5	25	16	100%	23.34	0.04	32.65	0.47
	MLL-AF4 e11e4	25	16	100%	23.36	0.05	34.49	1.05
	MLL-AF4 e11e5	25	16	100%	23.38	0.21	33.45	0.68

Accuracy

Cell lines (Invivoscribe) expressing fusion transcripts detected by the LEUKMPv2-RT24 kit were diluted to 1%-0.01% in the background of WT RNA and tested on a QuantStudio™ 5 and a CFX96.

QuantStudio™ 5 Accuracy Results

Clonal control RNA for target fusions was diluted in the background of lymphocytes RNA at 1:100 (1%), 1:1000 (0.1%) and 1:10000 (0.01%), at the minimum (25 ng) and maximum (250 ng) inputs. Each dilution was tested in 4 replicates on QuantStudio™ 5 instrument. Table below shows the dilutions at which the target fusion gene was detected in all replicates.

		250 ng				25 ng			
	Sample	Ref (VIC) Average Ct	Ref SD	Target Average Ct	Target SD	Ref (VIC) Average Ct	Ref SD	Target Average Ct	Target SD
WT	Lymphocytes Cell Line	22.05	0.05			25.63	0.08		
CBFβ-MYH11	ISV-0001	22.013	0.10	29.63	0.09			34.62	0.73
	ISV-0001.1	22.61	0.84	34.14	1.72	25.68	0.04	40.01	0.74
PML-RARA	ISV-0020	22.17	0.11	33.39	0.13	25.50	0.04	38.09	0.47
	ISV-0020.1	22.02	0.25	37.86	1.64	25.52	0.24	41.41	1.59
E2A-PBX1	ISV-0002	22.08	0.08	29.57	0.31	25.62	0.09	33.34	0.12
	ISV-0002.1	22.12	0.11	32.56	0.34	25.60	0.10	36.02	1.22