

One-Step Detection Kits for Fusion Transcripts

Real-Time PCR assay

- One-step cDNA synthesis
- 24 tests per kit
- Available as RUO (US) / IVD (EU)

LEUK1-QRT24 AML1-ETO
LEUK2-QRT24 E2A-PBX1
LEUK3-QRT24 MLL-AF4
LEUK5-QRT24 TEL-AML1
LEUK6-QRT24 CBFB-MYH11

INTENDED USE:

The One-Step Detection Kits for Fusion Transcripts are intended for the quantitative detection of the following fusion transcripts in bone marrow or peripheral blood samples.

t(8;21)(q22;q22) AML1-ETO

t(1;19)(q23;p13) E2A-PBX1

t(4;11)(q21;q23) MLL-AF4, 2 variants: e9-e4, e9-e5

t(12;21)(p13;q22) TEL-AML1

Inv(16) (p13q22) CBFB-MYH11, 3 variants: type A, D, E

PACKAGE CONTENTS

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

Description	Tubes
One-Step QRT-PCR Reaction Mix (5X)	1
Manganese Acetate	1
Molecular Grade Water	1
Fusion Transcript Detection Primer Mix	1
Fusion Transcript Positive Control	1

HANDLING AND STORAGE

This product is shipped on frozen ice packs and may be thawed upon arrival. Do not ship the product on dry ice. The contents of the shipment should be frozen at -25°C to -15°C immediately upon receipt. Do not store the kit or any of its components below -25°C as it will adversely affect the performance of the assay.

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

FOR USE WITH:

Validated on

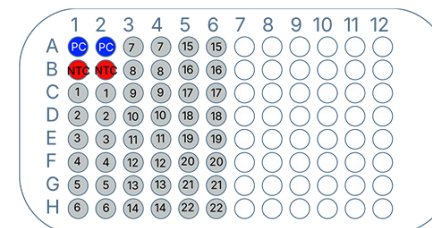
- Applied Biosystems® - 7500 Fast, StepOne
- Qiagen - Rotor-Gene® Q
- Roche - LightCycler® 480

Compatible with FAM/VIC dual color instruments including but not limited to:

- Applied Biosystems® - 7500, 7900HT, StepOne Plus
- BioRad - CFX96
- Qiagen - Rotor-Gene® 3000, 6000
- Roche - Cobas® 4800, Cobas® z480

PLATE SETUP

One kit contains enough reagents for 22 unknown samples, one positive control mix and one no template control (NTC).



ASSAY PERFORMANCE

The analytical performance of this assay was assessed on an Applied Biosystems® StepOne instrument synthetic long RNA sequences encoding each breakpoint region of the respective fusion genes, as well as RNA samples previously assessed for presence of the fusion genes.

Inter-assay Analysis

Dilution standards were created by diluting synthetic long RNA sequences representing each fusion variant transcript into total RNA isolated from healthy donor lymphocytes. The tables below summarize the results from these analyses for each fusion gene detection assay.

AML1-ETO

Dilution	Target	Mean Ct	SD
2.4x10 ⁵	ABL	20.654	0.277
	AML1-ETO	21.446	0.34
2.4x10 ⁴	ABL	21.588	0.032
	AML1-ETO	24.556	0.466
2.4x10 ³	ABL	21.653	0.014
	AML1-ETO	27.689	0.171
2.4x10 ²	ABL	21.724	0.007
	AML1-ETO	31.072	0.1
2.4x10 ¹	ABL	21.691	0.126
	AML1-ETO	34.08	0.306

TEL-AML1

Dilution	Target	Mean Ct	SD
2.4x10 ⁵	ABL	21.677	0.057
	TEL-AML1	21.684	0.083
2.4x10 ⁴	ABL	21.793	0.057
	TEL-AML1	24.93	0.077
2.4x10 ³	ABL	21.713	0.075
	TEL-AML1	27.973	0.013
2.4x10 ²	ABL	21.632	0.072
	TEL-AML1	31.027	0.125
2.4x10 ¹	ABL	21.574	0.149
	TEL-AML1	34.342	0.374

E2A-PBX1

Dilution	Target	Mean Ct	SD
2.4x10 ⁵	ABL	22.119	0.05
	E2A-PBX1	20.932	0.053
2.4x10 ⁴	ABL	22.007	0.043
	E2A-PBX1	24.374	0.01
2.4x10 ³	ABL	21.724	0.092
	E2A-PBX1	27.47	0.083
2.4x10 ²	ABL	21.678	0.008
	E2A-PBX1	30.639	0.299
2.4x10 ¹	ABL	21.653	0.004
	E2A-PBX1	33.731	0.414

CBFb-MYH11

Dilution	Target	Mean Ct	SD
2.4x10 ⁵	ABL	21.532	0.004
	CBFb-MYH11	20.43	0.043
2.4x10 ⁴	ABL	21.1658	0.069
	CBFb-MYH11	24.232	0.208
2.4x10 ³	ABL	21.159	0.091
	CBFb-MYH11	27.634	0.199
2.4x10 ²	ABL	21.009	0.165
	CBFb-MYH11	30.711	0.238
2.4x10 ¹	ABL	21.114	0.087
	CBFb-MYH11	34.078	0.216

MLL-AF4

Dilution	Target	Mean Ct	SD
2.4x10 ⁴	ABL	21.88	0.079
	MLL-AF4	24.217	0.022
2.4x10 ³	ABL	21.851	0.072
	MLL-AF4	27.525	0.162
2.4x10 ²	ABL	21.618	0.048
	MLL-AF4	30.845	0.085
2.4x10 ¹	ABL	21.649	0.01
	MLL-AF4	34.157	0.293
2.4x10 ⁰	ABL	21.727	0.03
	MLL-AF4	38.08	0.214

False-Positive and False-Negative Rates

To assess the false-positive and false-negative rates of the assay, RNA isolated from known positive and negative samples were analyzed in duplicate over 3 days. The positive samples comprised 3 patient samples that were previously assessed for the presence of each fusion transcript. The negative samples comprised RNA isolated from healthy donor lymphocytes. The table below summarizes the results of this analysis.

False Positivity	False Negativity
Healthy donors	Pt Samples
0% (0/54)	0% (0/54)

CONTACT

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For research use (RUO) in the U.S

For in vitro diagnostic use (IVD) in the European Union