For life science research use only. Not for use in diagnostic procedures. For *in vitro* use only.

TIB MOLBIOL

LightMix® Kit *human ABL1*

Cat.-No. 40-0357-16

Kit with reagents for the detection of *human ABL1* cDNA using the
Roche Diagnostics LightCycler® 1.x / 2.0 / 480 / 480 II Instruments.

Lyophilized mix of primers and probes for a total of 96 reactions with a final volume of 20 µl each. **Store protected from light at room temperature (18-25°C), do NOT freeze!**

Instructions for use with the LightCycler® 1.x / 2.0 Instruments see pages 4-5
Instructions for use with the LightCycler® 480 Instruments see pages 6-7

1. Introduction

Transcription is highly regulated; altered transcript levels can be the consequence of a disease. The study of gene expression is a powerful tool in medical research. Measurements are normalized against the overall amount of mRNA using the expression level of reference or housekeeping genes.

Human Abelson Murine Leukemia Viral Oncogene Homolog 1 (*ABL1*) is expressed ubiquitously and represents a reference gene. The protooncogene *ABL1* tyrosine-specific protein kinase plays a key role in cell signaling processes (stress response, cell division, cell adhesion, cell differentiation). In Europe the *ABL1* gene was chosen as the preferred reference for the quantification of fusion gene transcripts in leukemic residual disease detection[1,2].

The LightMix® Kit *human ABL1* provides a fast, easy and accurate system to identify and quantify this target in a nucleic acid extract.

This LightMix® Kit is tested on the LightCycler® 1.x / 2.0 / 480 and 480 II Instruments with Roche 'LightCycler® FastStart DNA Master HybProbe'. An one-step RT PCR procedure was not tested.

[1] Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia - a Europe Against Cancer program. Gabert J. et al. Leukemia 17:2318-2357 (2003).

[2] Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using 'real-time' quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR) – a Europe Against Cancer program. Beillard E. et al. Leukemia 17:2474-2486 (2003).

2. Description

A 137 bp fragment of the *human ABL1* cDNA is amplified with specific primers. The resulting PCR product is analyzed with LightCycler® Red 640 labeled hybridization probes (detected in channel 640).

The supplied standard row allows to determine the linear range of the reaction and to estimate the quantity of the target sequence in unknown samples.

For use in LightCycler® 1.x Instruments with software version 3.5.3 read channel F2 instead of channel 640, channel F3 instead of channel 705 and channel F1 instead of channel 530 for detection. We recommend upgrading LightCycler® 1.x Instruments to software version 4.1.

3. Set contents

6 Vials with red caps containing premixed lyophilized primers and probes for 16 PCR reactions each of *human ABL1*

1 Standard row with 6 lyophilized cloned plasmid standards of *human ABL1* DNA from 10^1 to 10^6 target equivalents per reaction

1 Sealing foil for the standard row

4. Additional reagents and items required

Roche Diagnostics:

LightCycler® FastStart DNA Master HybProbe	Cat.-No. 03 003 248 001
High Pure RNA Isolation Kit	Cat.-No. 11 828 665 001
Transcriptor First Strand cDNA Synthesis Kit	Cat.-No. 04 379 012 001
LightCycler® Capillaries (20 µl) (LightCycler® 1.x / 2.0 Instruments)	Cat.-No. 04 929 292 001
LightCycler® 480 Multiwell Plate 384, white (LightCycler® 480 Instrument)	Cat.-No. 04 729 749 001
or LightCycler® 480 Multiwell Plate 96, white (LightCycler® 480 Instrument)	Cat.-No. 04 729 692 001

5. Product characteristics

PCR results are obtained within 50 minutes (50 cycles and melting curve) with the LightCycler® 1.x / 2.0 Instruments and within 85 minutes (50 cycles and melting curve) with the LightCycler® 480 Instruments.

Sensitivity
These reagents detect 10 copies of *human ABL1* cDNA using the Roche 'LightCycler® FastStart DNA Master HybProbe' with the LightCycler® 1.x / 2.0 / 480 Instruments (in an exemplary system, using cloned targets as reference).

Measuring range
The linear measuring range of the assay is 10^2 to 10^6 copies of *human ABL1* cDNA using the Roche 'LightCycler® FastStart DNA Master HybProbe' with the LightCycler® 1.x / 2.0 / 480 Instruments.

Storage and Stability

- Lyophilized reagents are stable for at least 3 months after shipment when stored protected from light at room temperature (18-25℃).
- **Do not freeze** lyophilized reagents.
- Dissolved reagents are stable for at least 5 days when stored protected from light and refrigerated (4℃).

6. Experimental protocol

The following procedure was developed for use with the LightCycler® 1.x / 2.0 / 480 Instruments.
Start programming before preparing the solutions. See the LightCycler® Instrument operator's manual for details.

Sample material: Use aqueous nucleic acid preparations (e.g. Roche Diagnostics 'High Pure RNA Isolation Kit' combined with Roche Diagnostics 'Transcriptor First Strand cDNA Synthesis Kit').

Negative control: Always run at least one no-template control (NTC) - replace the template DNA with water.

Positive control: Run a positive control - replace the template DNA with the provided control DNA.
For quantification always use a fresh solution prepared from the provided standard row (single use only).

6.1. Preparation of parameter-specific reagents (16 reactions):

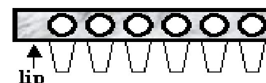
One reagent vial with a **red** cap contains all primers and probes to run 16 LightCycler® reactions for *human ABL1.*

Add 66 µl PCR-grade water to each reagent vial, mix the solution (vortex) and spin down.

►**Use 4 µl** reagent for a 20 µl PCR reaction.

| This solution is stable at least five days when stored refrigerated at 4°C. Avoid prolonged exposure t o light.

6.2. Preparation of the standard row



The target DNA is provided in 6 different quantities to yield from 10^1 to10^6 target molecules in 5 µl once resolved. Start with the lowest concentrated standard (first tube from the extended lip). Use the pipette tip to punch a hole through the sealing foil. **Add 40 µl** PCR-grade water to each vial of the row. Mix the target DNA by pipetting the solution up and down 10 times.

►**Use 5 µl** standard for a 20 µl PCR reaction.

This standard solution is not long-term stable and will lose sensitivity under prolonged storage. Use only fresh prepared solutions as quantification references. The standard row is intended for single use only.
After adding the target DNA to the LightCycler® reaction mix use the provided sealing foil to close the vials in order to avoid changes in the concentration due to evaporation.
Please note that opening of these vials may cause contaminations of the work-space (aerosol).

6.3. Preparation of the LightCycler® reaction mix

In a cooled reaction tube, prepare the reaction mix by multiplying each volume for a single reaction by the number of reactions to be cycled plus one additional reaction.

For use with the Roche FastStart Master	
Single reaction	**Component**
6.6 µl	water, PCR-grade (colorless cap, provided with the Roche Master kit)
2.4 µl	Mg^{2+} solution 25 mM (blue cap, provided with the Roche FastStart kit)
4.0 µl	**reagent** mix (parameter specific reagents containing primers and probes, see **6.1.**)
2.0 µl	Roche Master (red cap, for preparation see Roche manual)
15.0 µl	Volume of reaction mix

Mix gently, spin down and **transfer 15 µl** each of the reaction mix to a LightCycler® capillary (LightCycler® 1.x / 2.0 Instrument) or to a multiwell plate (LightCycler® 480 Instrument).

Add 5 µl of sample or standard to each capillary or well for a final reaction volume of 20 µl.

Start run.

7. LightCycler® 1.x / 2.0 Instruments

7.1. Programming

The protocol consists of four program steps

- 1: Denaturation: sample denaturation and enzyme activation
- 2: Cycling: PCR-amplification of the target DNA
- 3: Melting: melting curve analysis for identification of the PCR product derived from the target DNA
- 4: Cooling: cooling the instrument

Program Step:	Denaturation	Cycling			Melting			Cooling
Parameter								
Analysis Mode	None	Quantification mode			Melting Curves mode			None
Cycles	1	50			1			1
Target [℃]	95	95	60	72	95	40	85	40
Hold [hh:mm:ss]	00:10:00	00:00:05	00:00:10	00:00:20	00:00:20	00:00:20	00:00:00	00:00:30
Ramp Rate [℃/s]	20	20	20	20	20	20	0.2	20
Acquisition Mode	None	None	Single	None	None	None	Continuous	None

(Melting not relevant for detection)

Note: Unified run conditions for haematology parameters. No changes in reagents made. The former cycling program yields equivalent results.

7.2. Data Analysis

For use in LightCycler® 1.x Instruments select channel F2 instead of channel 640 for detection.

Perform data analysis, as described in the LightCycler® Instrument operator's manual.
We recommend using the Second Derivative Maximum method (Automated (F'' max)). The cycle number of the Crossing Point (CP) of each sample is calculated automatically. The Fit Points method is more-error prone due to the user's influence.

View *human ABL1* data in channel 640, Quantification mode. The negative control (NTC) must show no signal.

The provided standard row of cloned and purified DNA with concentrations in the range from 10^6 copies/rxn to 10^1 copies/rxn of *human ABL1* should have CPs between cycles 18 and 35 (CPs calculated with Second Derivative Maximum method).

7.3. Sample Data – typical results

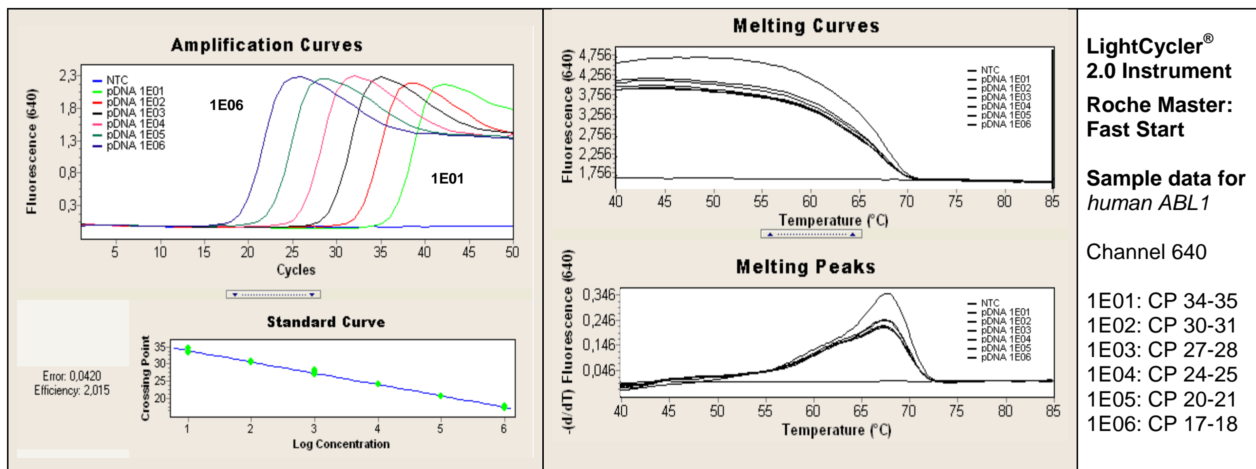


Fig.1. Sample data for the *human ABL1* detection system.

Data from LightCycler® 2.0 Instrument. Left panel channel 640 quantification mode (Second Derivative Maximum) with standard curve for *human ABL1*. Right panel channel 640 melting analysis for *human ABL1* (not relevant for detection).

Note: Fluorescence levels depend on instrument settings and may vary. The characteristics of the curve (shape and trend of fluorescence levels) should be similar to the curve in the manual. The fluorescent curves over cycles (quantification mode) must be smooth and not zigzag shaped. The CP values will vary from instrument to instrument by up to 2 cycles, while the distance between two dilution steps should be relative constant (delta CP). The CP values described in this manual (chart text) have been obtained using the supplied standard row; the entire set of curves can be horizontal shifted.

7.4. Interpretation of data

human ABL1 **(sample)**	**NTC**	**Result**
no amplification	negative	Negative
amplification signal	negative	Positive for *human ABL1*
amplification signal	positive	Contamination, repeat experiment

Tab. 3. Typical analysis results

8. LightCycler® 480 Instruments

8.1. Programming

The protocol consists of four program steps

- 1: Denaturation: sample denaturation and enzyme activation
- 2: Cycling: PCR-amplification of the target DNA
- 3: Melting: melting curve analysis for identification of the PCR product derived from the target DNA
- 4: Cooling: cooling the instrument

Detection Format:
LightCycler® 480 Instrument: 483-640
LightCycler® 480 II Instrument: 498-640

Program Step:	Denaturation	Cycling			Melting			Cooling
Parameter								
Analysis Mode	None	Quantification mode			Melting Curves mode			None
Cycles	1	50			1			1
Target [℃]	95	95	60	72	95	40	85	40
Hold [hh:mm:ss]	00:10:00	00:00:05	00:00:10	00:00:20	00:00:30	00:01:00	00:00:00	00:00:30
Ramp Rate [℃/s] **96**	4.4	4.4	2.2	4.4	4.4	1.5	-	1.5
Ramp Rate [℃/s] **384**	4.6	4.6	2.4	4.6	4.6	2.0	-	2.0
Acquisition Mode	None	None	Single	None	None	None	Continuous	None
Acquisitions [per ℃]	-	-	-	-	-	-	3	-

(Melting not relevant for detection)

8.2. Data Analysis

Perform data analysis, as described in the LightCycler® Instrument operator's manual.
We recommend using the Second Derivative Maximum method (Automated (F'' max)). The cycle number of the Crossing Point (CP) of each sample is calculated automatically. The Fit Points method is more-error prone due to the user's influence.

View *human ABL1* data with Filter Combination 483-640 Quantification mode. The negative control (NTC) must show no signal.

The provided standard row of cloned and purified DNA with concentrations in the range from 10^6 copies/rxn to 10^1 copies/rxn of *human ABL1* should have CPs between cycles 18 and 35 (CPs calculated with Second Derivative Maximum method).

8.3. Sample Data – typical results

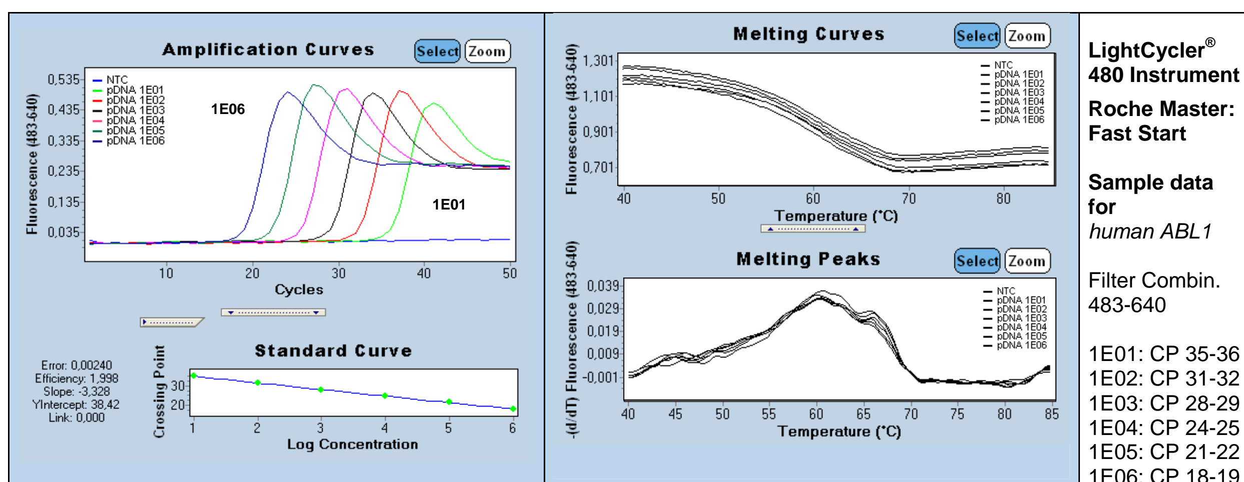


Fig.1. Sample data for the *human ABL1* detection system.

Data from LightCycler® 480 Instrument. Left panel filter combination 483-640 quantification mode (Second Derivative Maximum) with standard curve for *human ABL1*. Right panel filter combination 483-640 melting analysis for *human ABL1 (*not relevant for detection).

Note: Fluorescence levels depend on instrument settings and may vary. The characteristics of the curve (shape and trend of fluorescence levels) should be similar to the curve in the manual. The fluorescent curves over cycles (quantification mode) must be smooth and not zigzag shaped. The CP values will vary from instrument to instrument by up to 2 cycles, while the distance between two dilution steps should be relative constant (delta CP). The CP values described in this manual (chart text) have been obtained using the supplied standard row; the entire set of curves can be horizontal shifted.

8.4. Interpretation of data

***human ABL1* (sample)**	**NTC**	**Result**
no amplification	negative	Negative
amplification signal	negative	Positive for *human ABL1*
amplification signal	positive	Contamination, repeat experiment

Tab. 3. Typical analysis results

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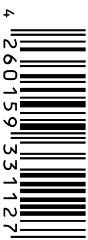

These reagents were developed and manufactured by TIB MOLBIOL GmbH, Berlin, Germany.
LightCycler® hybridization probes produced under license from Roche Diagnostics GmbH.