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ipsogen® BCR-ABL1 Mbcr Kit Handbook

Σ 24

Version 1

IVD

Quantitative in vitro diagnostics

For use with Rotor-Gene® Q, ABI PRISM®, LightCycler®, and
SmartCycler® instruments

CE

REF 670123

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Contents

Intended Use

The *ipsogen* BCR-ABL1 Mbcr Kit is intended for the quantification of BCR-ABL p210 b2a2 or b3a2 transcripts in bone marrow or peripheral blood samples of acute lymphoblastic leukemia (ALL) or chronic myeloid leukemia (CML) patients previously diagnosed with a BCR-ABL Mbcr fusion gene (FG) event. The test is intended to evaluate the level of molecular response; results can be used for minimal residual disease follow-up.

Summary and Explanation

CML belongs to the group of myeloproliferative neoplasms and is in >90% of cases characterized by the presence of the Philadelphia chromosome (Ph CHRS).

This chromosome is the product of a reciprocal translocation between the long arms of chromosomes 9 and 22, t(9;22), BCR (breakpoint cluster region) being located on chromosome 22 and the c-ABL oncogene coming from chromosome 9. The corresponding fusion gene, BCR-ABL, is transcribed into an 8.5 kb mRNA, with 2 junction variants b2a2 (40% of cases) and b3a2 (55% of cases). It encodes a chimeric protein, p210, with elevated tyrosine kinase activity. The b2a3 and b3a3 transcripts represent less than 5% of cases. A Ph chromosome can also be detected in 35% of adult ALL patients.

Annual incidence of CML is approximately 1–2 per 100,000, and CML accounts for 20% of adult leukemias. It is characterized clinically by an excess of myeloid cells that differentiate and function normally. CML patients will be diagnosed in 90–95% of cases in the chronic or stable phase of the disease. Historically, within an average of 4 to 6 years, patients were entering an accelerated phase leading to blastic crisis and acute leukemia, which is always fatal. The advents of imatinib and more recently, second generation tyrosine kinase inhibitors (TKI), dramatically changed the natural course of the disease: most of the patients now remain in remission and deserve long-term follow-up and disease monitoring.

Disease monitoring

To date, the goal of CML therapy is to achieve 100% survival and Ph chromosome negativity. Disease monitoring is therefore an essential tool to assess treatment response and detect early relapse for each individual patient. Under TKI therapy, patients typically progress from hematologic to cytogenetic then molecular remission, corresponding to decreasing numbers of leukemic cells and BCR-ABL transcripts as detailed in the Figure 1 below.

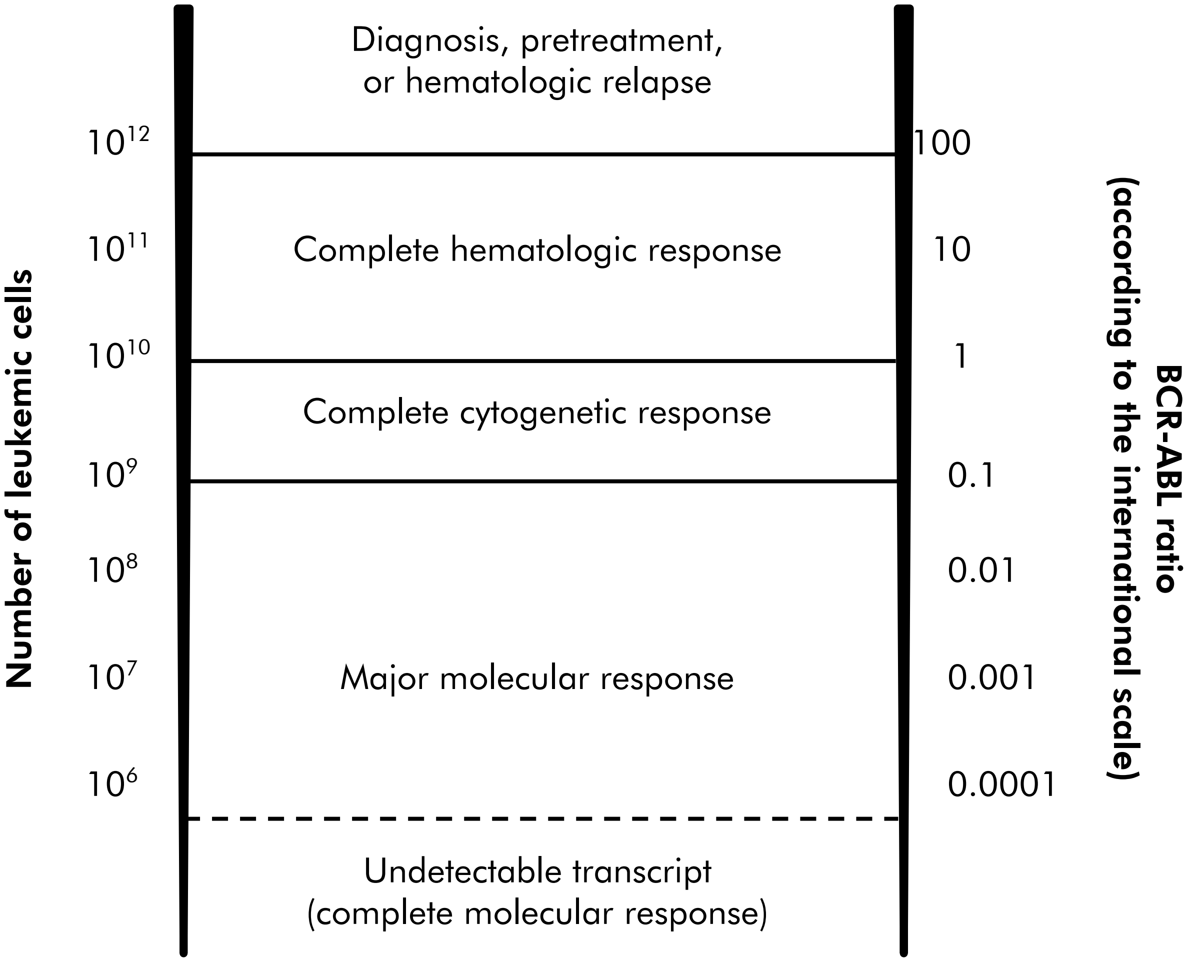

Figure 1. Adapted from reference 1.

The standard method to estimate the tumor burden in CML patients is conventional cytogenetic analysis (G-banding) on bone marrow (BM) metaphases. Cytogenetic response is assessed on at least 20 marrow metaphases. The level of cytogenetic response is estimated on the percentage of Ph chromosome-positive metaphases (see Table 1, reference 2) However, this assessment depends on laboratory performances and has a low sensitivity, at 5% when 20 metaphases are analyzed.

Real time quantitative polymerase chain reaction (qPCR) quantifying BCR-ABL Mbcr mRNA, on peripheral blood (PB) specimens is now part of the disease monitoring techniques for CML on treatment. It is less invasive than conventional bone marrow metaphase cytogenetics, and more sensitive.

Recommendations for CML disease monitoring have also been recently updated to incorporate new clinical evidence from clinical trials as well as improved disease monitoring objectives and tools. The most recent recommendations on response definition and monitoring of patients on imatinib come from the ELN experts (2).

From a technical standpoint, efforts have been made by international experts to harmonize BCR-ABL Mbcr testing and reporting (3–5). Additionally, a reference panel has been validated recently under auspices of the WHO, to allow a simple standardization of BCR-ABL quantification (6).

Table 1. International recommendations for the management of CML patients (adapted from reference 2)

	Hematologic response	**Cytogenetic response**	**Molecular response (BCR-ABL to control gene ratio according to the international scale)**
Definitions	Complete: Platelet count <450 x 10^9/liter White blood cell count <10 x 10^9/liter Differential without immature granulocytes and with less than 5% basophils Non-palpable spleen	Complete: Ph+ 0% Partial: Ph+ 1–35% Minor: Ph+ 36–65% Minimal: Ph+ 66–95% None: Ph+ >95%	"Complete" indicates transcript nonquantifiable and nondetectable Major: ≤0.1
Monitoring	Check every 2 weeks until complete response is achieved and confirmed, then 3 monthly unless otherwise required	Check at least every 6 months until complete response is achieved and confirmed, then at least every 12 months	Check every 3 months Mutational analysis in case of failure, suboptimal response or transcript level increase

Complete hematologic response, cytogenetic response and molecular response should be confirmed on two subsequent occasions. Cytogenetic response is evaluated by morphologic cytogenetics of at least 20 marrow metaphases. Fluorescence in situ hybridization (FISH) of peripheral blood cells should be used only if marrow cells cannot be obtained. Molecular response is assessed on peripheral blood cells.

Principle of the Procedure

qPCR permits the accurate quantification of PCR products during the exponential phase of the PCR amplification process. Quantitative PCR data can be rapidly obtained, without post-PCR processing, by real-time detection of fluorescent signals during and/or subsequent to PCR cycling, thereby drastically reducing the risk of PCR product contamination. At present, 3 main types of qPCR techniques are available: qPCR analysis using SYBR® Green I Dye, qPCR analysis using hydrolysis probes, and qPCR analysis using hybridization probes.

This assay exploits the qPCR double-dye oligonucleotide hydrolysis principle. During PCR, forward and reverse primers hybridize to a specific sequence (Figure 2). A double-dye oligonucleotide is contained in the same mix. This probe, which consists of an oligonucleotide labeled with a 5' reporter dye and a downstream, 3'quencher dye, hybridizes to a target sequence within the PCR product. qPCR analysis with hydrolysis probes exploits the 5'→3' exonuclease activity of the *Thermus aquaticus* (*Taq*) DNA polymerase. When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence primarily by Förster-type energy transfer.

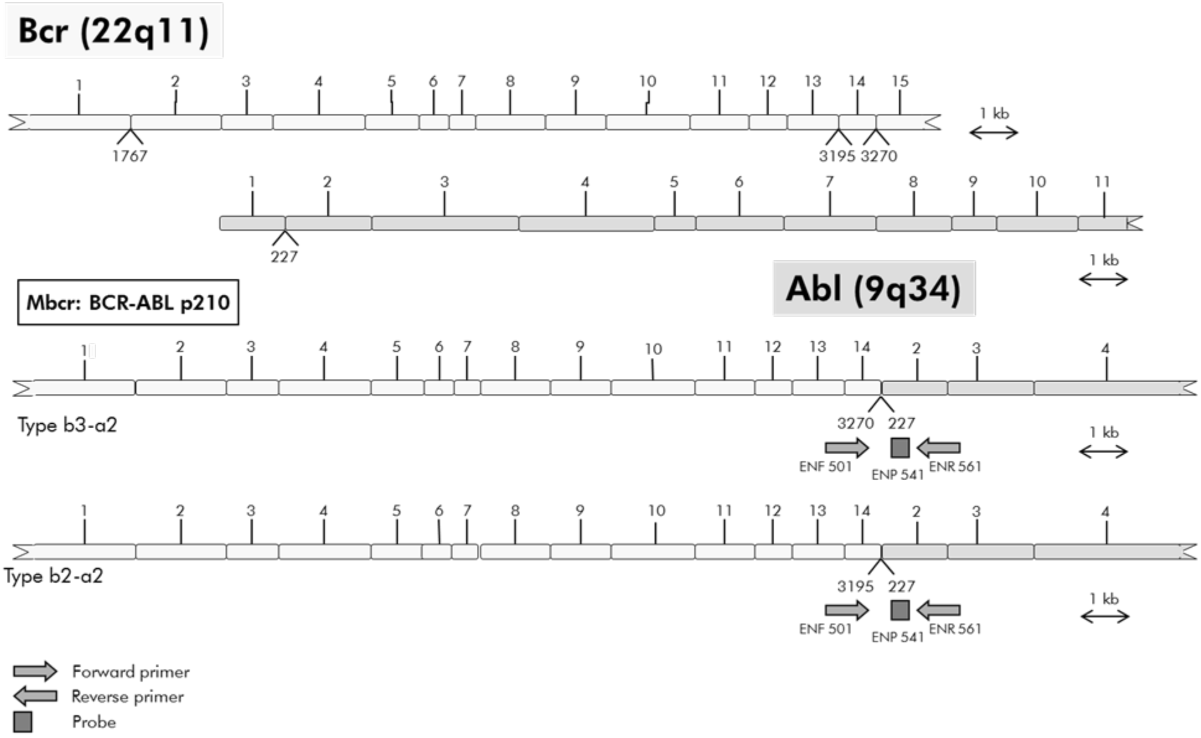

Figure 2. Schematic diagram of the BCR-ABL Mbcr FG transcript covered by the qPCR primers and probe set: ENF501–ENP541–ENR561. The number under the primers and probe refers to their nucleotide position in the normal gene transcript.

During PCR, if the target of interest is present, the probe specifically anneals between the forward and reverse primer sites. The 5'→3' exonuclease activity of the DNA polymerase cleaves the probe between the reporter and the quencher only if the probe hybridizes to the target. The probe fragments are then displaced from the target, and polymerization of the strand continues. The 3' end of the probe is blocked to prevent extension of the probe during PCR (Figure 3). This process occurs in every cycle and does not interfere with the exponential accumulation of product.

The increase in fluorescence signal is detected only if the target sequence is complementary to the probe and hence amplified during PCR. Because of these requirements, nonspecific amplification is not detected. Thus, the increase in fluorescence is directly proportional to the target amplification during PCR.

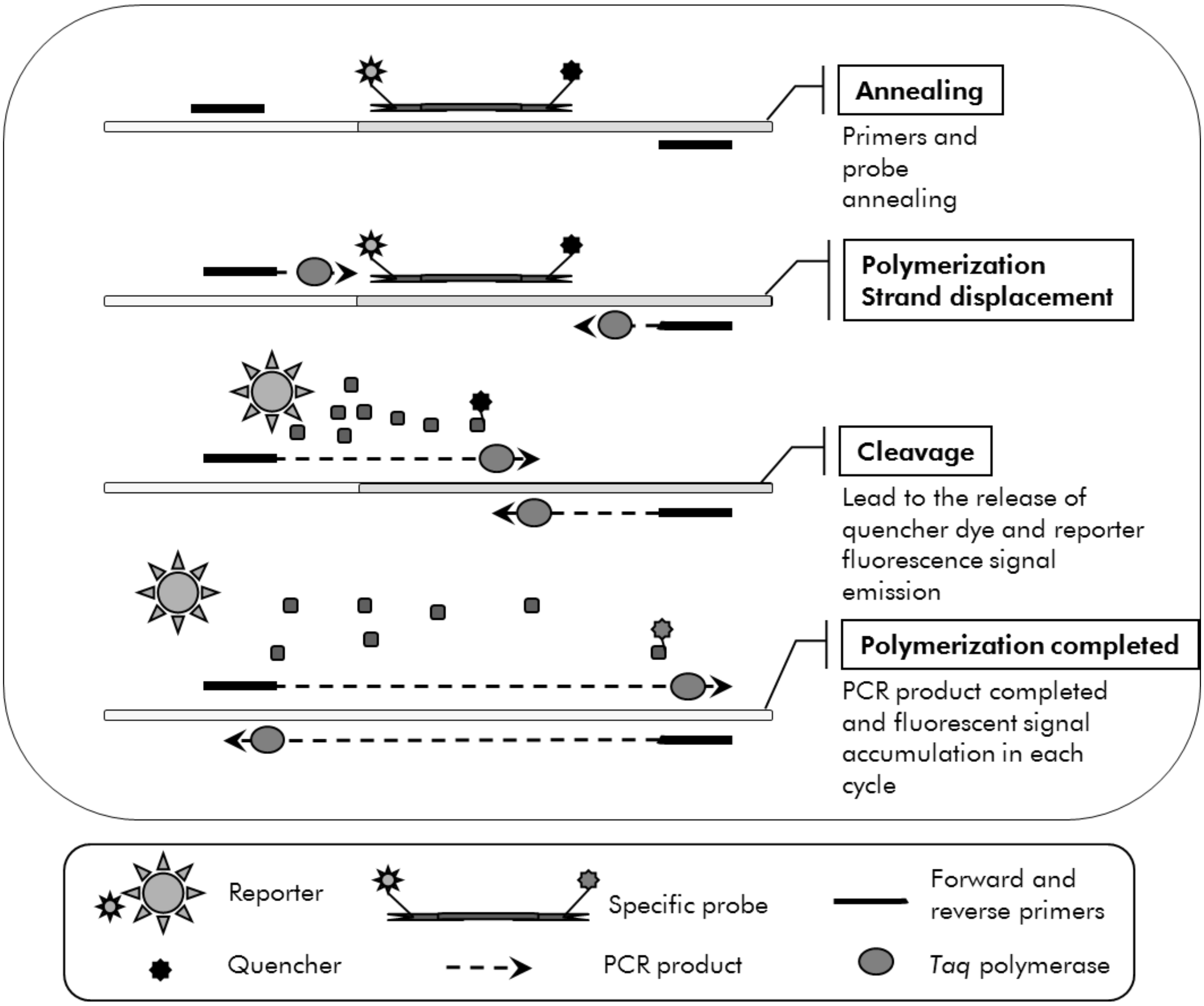

Figure 3. Reaction principle. Total RNA is reverse-transcribed, and the generated cDNA is amplified by PCR using a pair of specific primers and a specific internal double-dye probe (FAM™–TAMRA™). The probe binds to the amplicon during each annealing step of the PCR. When the *Taq* DNA polymerase extends from the primer bound to the amplicon, it displaces the 5' end of the probe, which is then degraded by the 5'→3' exonuclease activity of the *Taq* DNA polymerase. Cleavage continues until the remaining probe melts off the amplicon. This process releases the fluorophore and quencher into solution, spatially separating them and leading to an increase in fluorescence from the FAM and a decrease in fluorescence from the TAMRA.

Materials Provided

Kit contents

***ipsogen* BCR-ABL1 Mbcr Kit**		**(24)**
Catalog no.		**670123**
Number of reactions		**24**
ABL Control Gene Standard Dilution (10^3 copies/5 μl)	C1-ABL	50 μl
ABL Control Gene Standard Dilution (10^4 copies/5 μl)	C2-ABL	50 μl
ABL Control Gene Standard Dilution (10^5 copies/5 μl)	C3-ABL	50 μl
BCR-ABL Mbcr Fusion Gene Standard Dilution (10^1 copies/5 μl)	F1-BCR-ABL Mbcr	50 μl
BCR-ABL Mbcr Fusion Gene Standard Dilution (10^2 copies/5 μl)	F2-BCR-ABL Mbcr	50 μl
BCR-ABL Mbcr Fusion Gene Standard Dilution (10^3 copies/5 μl)	F3-BCR-ABL Mbcr	50 μl
BCR-ABL Mbcr Fusion Gene Standard Dilution (10^5 copies/5 μl)	F4-BCR-ABL Mbcr	50 μl
BCR-ABL Mbcr Fusion Gene Standard Dilution (10^6 copies/5 μl)	F5-BCR-ABL Mbcr	50 μl
Primers and Probe Mix ABL*	PPC-ABL 25x	90 μl
Primers and Probe Mix BCR-ABL Mbcr Fusion Gene†	PPF-Mbcr 25x	110 μl
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* Mix of specific reverse and forward primers for the ABL control gene plus a specific FAM–TAMRA probe.

† Mix of specific reverse and forward primers for the BCR-ABL Mbcr fusion gene plus a specific FAM–TAMRA probe.

Note: Briefly centrifuge the standard dilutions and the primers and probe mixes before use.

Materials Required but Not Provided

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Reagents

- Nuclease-free PCR grade water
- Reagents for reverse transcription: The validated reagent is Superscript® II (or Superscript) Reverse Transcriptase, includes 5x first-strand buffer, 100 mM DTT (Life Technologies, cat. no. 18064-022)
- RNase inhibitor: The validated reagent is RNaseOUT™ (Life Technologies, cat. no. 10777-019)
- Set of dNTPs, PCR grade
- Random hexamer
- $MgCl_2$
- Buffer and *Taq* DNA polymerase: The validated reagents are TaqMan® Universal PCR Master Mix (Master Mix PCR 2x) (Life Technologies, cat. no. 4304437) and LightCycler TaqMan Master (Master Mix PCR 5x) (Roche, cat. no. 04535286001)

Consumables

- Nuclease-free aerosol-resistant sterile PCR pipet tips with hydrophobic filters
- 0.5 ml or 0.2 ml RNase- and DNase-free PCR tubes
- Ice

Equipment

- Microliter pipet* dedicated for PCR (1–10 µl; 10–100 µl; 100–1000 µl)
- Benchtop centrifuge* with rotor for 0.2 ml/0.5 ml reaction tubes (capable of attaining 10,000 rpm)
- Real-time PCR instrument:* Rotor-Gene Q MDx 5plex HRM or other Rotor-Gene instrument; LightCycler 1.2, 2.0, or 480; ABI PRISM 7000, 7700, or 7900HT SDS; or SmartCycler instrument; and associated specific material
- Thermal cycler* or water bath* (reverse transcription step)

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* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Complementary reagents

- *ipsogen* BCR-ABL1 Mbcr Controls Kit (cat. no. 670191), consisting of cell lines with negative, high, and low positive expression of the BCR-ABL Mbcr fusion gene for the qualitative validation of the RNA extraction and the reverse transcription

Warnings and Precautions

For in vitro diagnostic use

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Discard sample and assay waste according to your local safety regulations.

General precautions

qPCR tests require good laboratory practices, including equipment maintenance, that are dedicated to molecular biology and compliant with applicable regulations and relevant standards.

This kit is intended for in vitro diagnostic use. Reagents and instructions supplied in this kit have been validated for optimal performance. Further dilution of the reagents or alteration of incubation times and temperatures may result in erroneous or discordant data. PPC and PPF reagents may be altered if exposed to light. All reagents are formulated specifically for use with this test. For optimal performance of the test, no substitutions should be made.

Determining transcript levels using qPCR requires both the reverse transcription of the mRNA and the amplification of the generated cDNA by PCR. Therefore, the entire assay procedure must be performed under RNase-/DNase-free conditions.

Use extreme caution to prevent:

- RNase/DNase contamination, which might cause degradation of the template mRNA and the generated cDNA
- mRNA or PCR carryover contamination resulting in false positive signal

We therefore recommend the following.

- Use nuclease-free labware (e.g., pipets, pipet tips, reaction vials) and wear gloves when performing the assay.
- Use fresh aerosol-resistant pipet tips for all pipetting steps to avoid cross-contamination of the samples and reagents.

- Prepare pre-PCR master mix with dedicated material (pipets, tips, etc.) in a dedicated area where no DNA matrixes (cDNA, DNA, plasmid) are introduced. Add template in a separate zone (preferably in a separate room) with specific material (pipets, tips, etc.).
- Handle the standard dilutions (C1–3 and F1–5) in a separate room.

Reagent Storage and Handling

The kits are shipped on dry ice and must be stored at –30°C to –15°C upon receipt.

- Minimize exposure to light of the primers and probe mixes (PPC and PPF tubes).
- Gently mix and centrifuge the tubes before opening.
- Store all kit components in original containers.

These storage conditions apply to both opened and unopened components. Components stored under conditions other than those stated on the labels may not perform properly and may adversely affect the assay results.

Expiration dates for each reagent are indicated on the individual component labels. Under correct storage conditions, the product will maintain performance until the expiration date printed on the label.

There are no obvious signs to indicate instability of this product. However, positive and negative controls should be run simultaneously with unknown specimens.

Procedure

Sample RNA preparation

RNA preparation from patient samples (blood or bone marrow) must have been performed with a validated procedure. The quality of the assay is largely dependent on the quality of input RNA. We therefore recommend qualifying the purified RNA by agarose* gel electrophoresis or by using Agilent® Bioanalyzer® prior to analysis.

Protocol: Recommended standardized EAC reverse transcription

Things to do before starting

- Prepare dNTPs, 10 mM each. Store at –20°C in aliquots.

Procedure

1. **Thaw all necessary components and place them on ice.**
2. **Incubate 1 µg of RNA (1–4 µl) for 10 minutes at 70°C and immediately cool on ice for 5 minutes.**
3. **Centrifuge briefly (approximately 10 seconds, 10,000 rpm, to collect the liquid in the bottom of the tube). Then keep on ice.**
4. **Prepare the following RT mix according to the number of samples being processed (Table 2).**

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* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

Table 2. Preparation of RT mix

Component	Volume per sample (µl)	Final concentration
First-Strand Buffer (supplied with Superscript II Reverse Transcriptase), 5x	4.0	1x
$MgCl_2$ (50 mM)	2.0	5 mM
dNTPs (10 mM each, to be prepared previously and stored at –20°C in aliquots)	2.0	1 mM
DTT (100 mM, supplied with Superscript II Reverse Transcriptase)	2.0	10 mM
RNase inhibitor (40 U/µl)	0.5	1 U/µl
Random hexamer (100 µM)	5.0	25 µM
Superscript II or Superscript Reverse Transcriptase (200 U/µl)	0.5	5 U/µl
Heated RNA sample (to be added in step 5)	1.0–4.0	50 ng/µl
Nuclease-free PCR grade water (to be added in step 5)	0.0–3.0	–
Final volume	20.0	–

5. **Pipet 16 µl of RT mix into each PCR tube. Then add 1–4 µl (1 µg) RNA (from step 3), and adjust the volume to 20 µl with nuclease-free PCR grade water (see Table 3).**

Table 3. Preparation of reverse transcription reaction

Component	Volume (µl)
RT mix	16
Heated sample RNA (1 µg)	1–4
Nuclease-free PCR grade water	0–3
Final volume	20

6. **Mix well and centrifuge briefly (approximately 10 seconds, 10,000 rpm, to collect the liquid in the bottom of the tube).**
7. **Incubate at 20°C for 10 minutes.**
8. **Incubate at 42°C on a thermal cycler for 45 minutes, then immediately at 99°C for 3 minutes.**
9. **Cool on ice (to stop the reaction) for 5 minutes.**
10. **Briefly centrifuge (approximately 10 seconds, 10,000 rpm, to collect the liquid in the bottom of the tube). Then keep on ice.**
11. **Dilute the final cDNA with 30 µl of nuclease-free PCR grade water so that the final volume is 50 µl.**
12. **Carry out PCR according to the following protocols, according to your qPCR instrument.**

Protocol: qPCR on Rotor-Gene Q MDx 5plex HRM or Rotor-Gene Q 5plex HRM instruments with 72-tube rotor

Using this instrument, we recommend performing all measurements in duplicate, as indicated in Table 4.

Table 4. Number of reactions for Rotor-Gene Q instruments with 72-tube rotor

Samples	Reactions
With the ABL primers and probe mix (PPC-ABL)	
n cDNA samples	n x 2 reactions
ABL standard	2 x 3 reactions (3 dilutions, each one tested in duplicate)
Water control	2 reactions
With the BCR-ABL Mbcr primers and probe mix (PPF-Mbcr)	
n cDNA samples	n x 2 reactions
Mbcr standard	2 x 5 reactions (5 dilutions, each one tested in duplicate)
Water control	2 reactions

Sample processing on Rotor-Gene Q instruments with 72-tube rotor

We recommend testing at least 8 cDNA samples in the same experiment to optimize the use of the standards and the primers and probe mixes. Each *ipsogen* BCR-ABL1 Mbcr Kit provides enough reagents to perform an 8-sample experiment 3 times using the 72-tube rotor.

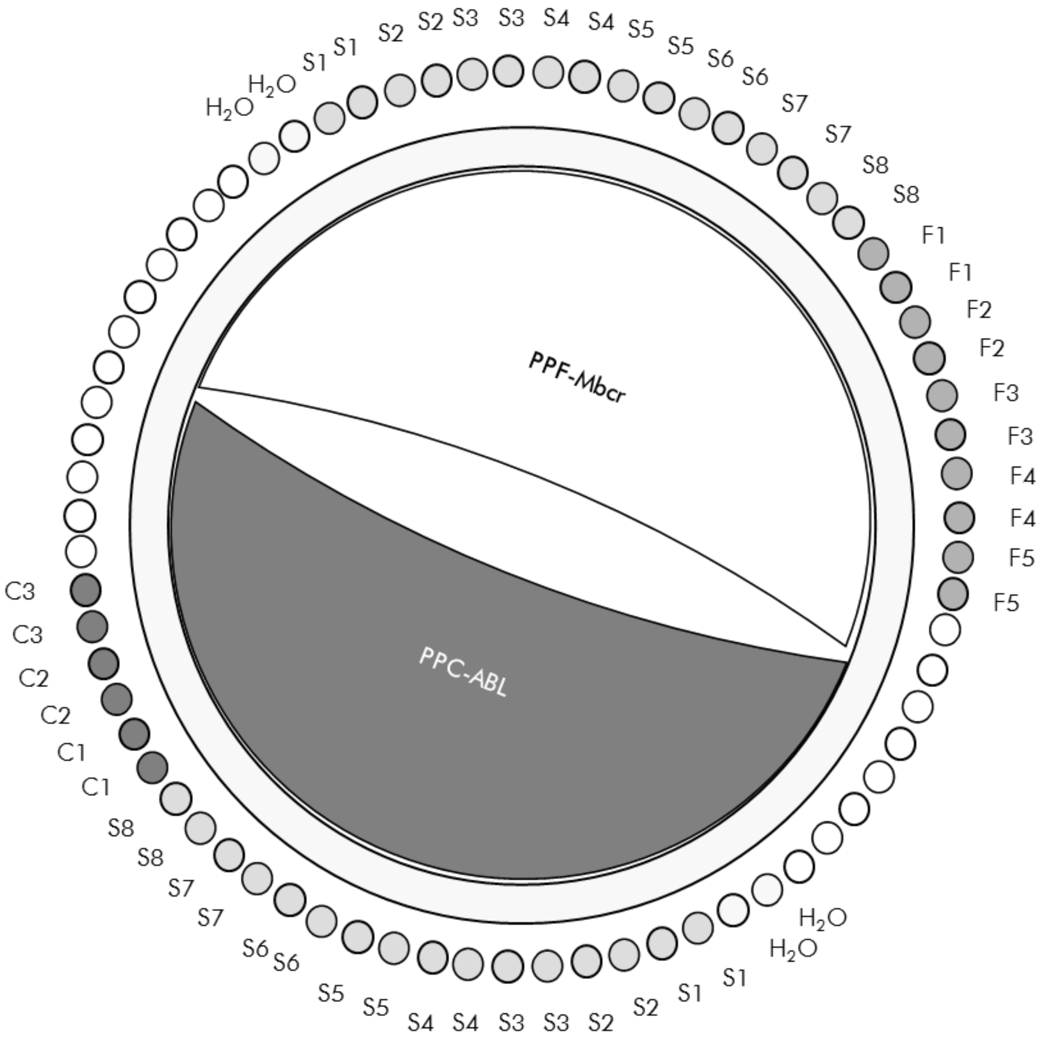


Figure 4. Suggested rotor setup for each experiment with the *ipsogen* BCR-ABL1 Mbcr Kit. F1–5: BCR-ABL Mbcr standards; **C1–3**: ABL standards; **S**: cDNA sample; **H_2O**: water control.

Note: Take care to always place a sample to be tested in position 1 of the rotor. Otherwise, during the calibration step, the instrument will not perform calibration, and incorrect fluorescence data will be acquired.

Fill all other positions with empty tubes.

qPCR on Rotor-Gene Q instruments with 72-tube rotor

Note: Perform all steps on ice.

Procedure

1. **Thaw all necessary components and place them on ice.**
2. **Prepare the following qPCR mix according to the number of samples being processed.**

   All concentrations are for the final volume of the reaction.

Table 5 describes the pipetting scheme for the preparation of one reagent mix, calculated to achieve a final reaction volume of 25 µl. A pre-mix can be prepared, according to the number of reactions, using the same primer and probe mix (either PPC-ABL or PPF-Mbcr). Extra volumes are included to compensate for pipetting error.

Table 5. Preparation of qPCR mix

Component	1 reaction (µl)	ABL: 24+1 reactions (µl)	BCR-ABL Mbcr: 28+1 reactions (µl)	Final concentration
TaqMan Universal PCR Master Mix, 2x	12.5	312.5	362.5	1x
Primers and probe mix, 25x	1	25	29	1x
Nuclease-free PCR grade water	6.5	162.5	188.5	–
Sample (to be added at step 4)	5	5 each	5 each	–
Total volume	25	25 each	25 each	–

3. **Dispense 20 µl of the qPCR pre-mix per tube.**
4. **Add 5 µl of the RT product (cDNA, 100 ng RNA equivalent) obtained in the reverse transcription (see "Protocol: Recommended standardized EAC reverse transcription", page 14) in the corresponding tube (total volume 25 µl).**
5. **Mix gently, by pipetting up and down.**
6. **Place the tubes in the thermal cycler according to the manufacturer recommendations.**
7. **Program the Rotor-Gene Q instrument with the thermal cycling program as indicated in Table 6.**

Table 6. Temperature profile

Mode of analysis	Quantitation
Hold	Temperature: 50 deg Time: 2 mins
Hold 2	Temperature: 95 deg Time: 10 mins
Cycling	50 times 95 deg for 15 secs 60 deg for 1 min with acquisition of FAM fluorescence in channel Green: Single

8. **For Rotor-Gene Q instruments, select "Slope Correct" for the analysis. We recommend setting the threshold at 0.03. Start the thermal cycling program, as indicated in Table 6.**

Protocol: qPCR on ABI PRISM 7000, 7700, and 7900HT SDS, and LightCycler 480 Instrument

Using 96-well-plate qPCR equipment, we recommend performing all measurements in duplicate, as indicated in Table 7.

Table 7. Number of reactions using 96-well-plate qPCR equipment

Samples	Reactions
With the ABL primers and probe mix (PPC-ABL)	
n cDNA samples	n x 2 reactions
ABL standard	2 x 3 reactions (3 dilutions, each one tested in duplicate)
Water control	2 reactions
With the BCR-ABL Mbcr primers and probe mix (PPF-Mbcr)	
n cDNA samples	n x 2 reactions
Mbcr standard	2 x 5 reactions (5 dilutions, each one tested in duplicate)
Water control	2 reactions

Sample processing on ABI PRISM 7000, 7700, and 7900 SDS, and LightCycler 480 Instrument

We recommend testing at least 8 cDNA samples in the same experiment to optimize the use of the standards and the primers and probe mixes. The plate scheme in Figure 5 shows an example of such an experiment.

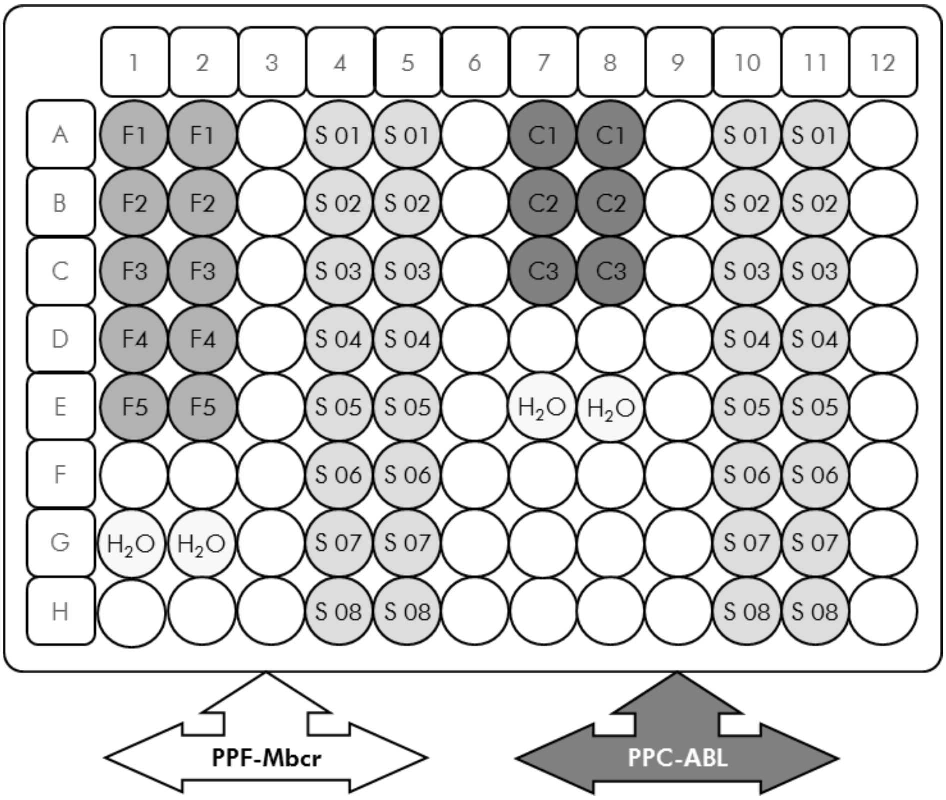


Figure 5. Suggested plate setup for one experiment. S: cDNA sample; **F1–5**: BCR-ABL Mbcr standards; **C1–3**: ABL standards; **H_2O**: water control.

qPCR on ABI PRISM 7000, 7700, and 7900 SDS, and LightCycler 480 Instrument

Note: Perform all steps on ice.

Procedure

1. **Thaw all necessary components and place them on ice.**
2. **Prepare the following qPCR mix according to the number of samples being processed. If using 96-well-plate qPCR equipment, we recommend performing all measurements in duplicate.**

   All concentrations are for the final volume of the reaction.

   Table 8 describes the pipetting scheme for the preparation of one reagent mix, calculated to achieve a final reaction volume of 25 µl. A pre-mix can be prepared, according to the number of reactions, using the same primer and probe mix (either PPC-ABL or PPF-Mbcr). Extra volumes are included to compensate for pipetting error.

Table 8. Preparation of qPCR mix

Component	1 reaction (µl)	ABL: 24+1 reactions (µl)	BCR-ABL Mbcr: 28+1 reactions (µl)	Final concentration
TaqMan Universal PCR Master Mix, 2x	12.5	312.5	362.5	1x
Primers and probe mix, 25x	1	25	29	1x
Nuclease-free PCR grade water	6.5	162.5	188.5	–
Sample (to be added at step 4)	5	5 each	5 each	–
Total volume	25	25 each	25 each	–

3. **Dispense 20 µl of the qPCR pre-mix per well.**
4. **Add 5 µl of the RT product (cDNA, 100 ng RNA equivalent) obtained in the reverse transcription (see "Protocol: Recommended standardized EAC reverse transcription", page 14) in the corresponding well (total volume 25 µl).**
5. **Mix gently, by pipetting up and down.**
6. **Close the plate and briefly centrifuge (300 x *g*, approximately 10 seconds).**
7. **Place the plate in the thermal cycler according to the manufacturer recommendations. Program the thermal cycler with the thermal cycling program as indicated in Table 9 for ABI PRISM 7000, 7700, and 7900HT SDS, or Table 10 for the LightCycler 480 Instrument.**

Table 9. Temperature profile for ABI PRISM 7000, 7700, and 7900HT SDS

Mode of analysis	Standard Curve — Absolute Quantitation
Hold	Temperature: 50°C Time: 2 minutes
Hold 2	Temperature: 95°C Time: 10 minutes
Cycling	50 times 95°C for 15 seconds 60°C for 1 minute with acquisition of FAM fluorescence; quencher: TAMRA

Table 10. Temperature profile for LightCycler 480 Instrument

Mode of analysis	Absolute Quantification (“Abs Quant”)
Detection formats	Select “Simple Probe” in the Detection formats window
Hold	Temperature: 50°C Time: 2 minutes
Hold 2	Temperature: 95°C Time: 10 minutes
Cycling	50 times 95°C for 15 seconds 60°C for 1 minute with acquisition of FAM fluorescence corresponding to (483–533 nm) for LC version 01 and (465–510 nm) for LC version 02

8. **For the ABI PRISM 7000, 7700, and 7900HT SDS, follow step 8a. For the LightCycler 480 Instrument, follow step 8b.**

8a. ABI PRISM 7000, 7700. and 7900HT SDS: We recommend a threshold set at 0.1 as described in the EAC protocol in the analysis step on the ABI PRISM SDS and a baseline set between cycles 3 and 15. Start the cycling program, as indicated in Table 9.

8b. LightCycler 480 Instrument: We recommend a Fit point analysis mode with background at 2.0 and threshold at 2.0. Start the thermal cycling program, as indicated in Table 10.

Protocol: qPCR on LightCycler 1.2 and 2.0 Instruments

Using capillary instruments, we recommend measuring samples in duplicate and controls only once, as indicated in Table 11.

Table 11. Number of reactions for LightCycler 1.2 and 2.0 Instruments

Samples	Reactions
With the ABL primers and probe mix (PPC-ABL)	
n cDNA samples	n x 2 reactions
ABL standard	1 x 3 reactions (3 standard dilutions, each one tested once)
Water control	1 reaction
With the BCR-ABL Mbcr primers and probe mix (PPF-Mbcr)	
n cDNA samples	n x 2 reactions
Mbcr standard	1 x 5 reactions (5 standard dilutions, each one tested once)
Water control	1 reaction

Sample processing on LightCycler 1.2 and 2.0 Instruments

We recommend testing at least 5 cDNA samples in the same experiment to optimize the use of the standards and primers and probe mixes. The capillary scheme in Figure 6 shows an example of an experiment.

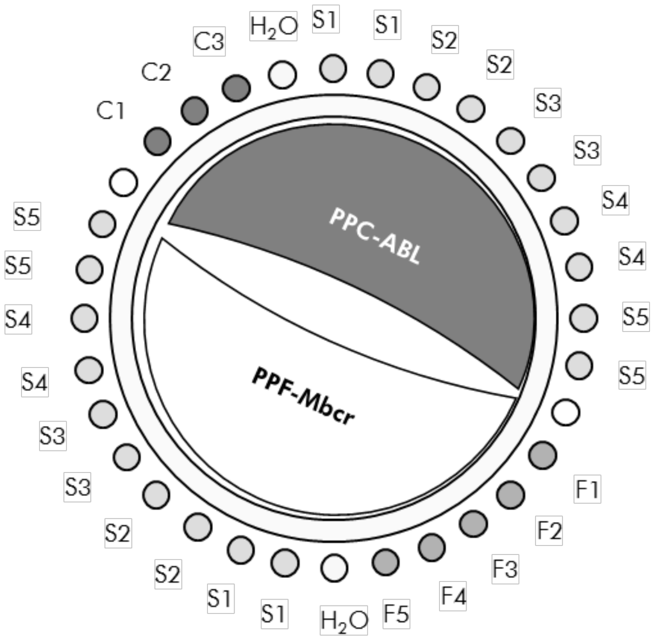


Figure 6. Suggested rotor setup for each experiment with the *ipsogen* BCR-ABL1 Mbcr Kit. F1–5: BCR-ABL Mbcr standards; **C1–3**: ABL standards; **S**: unknown DNA sample to be analyzed; **H_2O**: water control.

qPCR on LightCycler 1.2 and 2.0 Instruments

Note: Because of particular technological requirements, LightCycler experiments must be performed using specific reagents. We recommend to use the LightCycler TaqMan Master and to follow the manufacturer's instructions to prepare the Master Mix 5x.

Note: Perform all steps on ice.

Procedure

1. **Thaw all necessary components and place them on ice.**
2. **Prepare the following qPCR mix according to the number of samples being processed.**

   All concentrations are for the final volume of the reaction.

   Table 12 describes the pipetting scheme for the preparation of one reagent mix, calculated to achieve a final reaction volume of 20 µl. A pre-mix can be prepared, according to the number of reactions, using the same primer and probe mix (either PPC-ABL or PPF-Mbcr). Extra volumes are included to compensate for pipetting error.

Table 12. Preparation of qPCR mix

Component	1 reaction (µl)	ABL: 14+1 reactions (µl)	BCR-ABL Mbcr: 16+1 reactions (µl)	Final concentration
Freshly prepared LightCycler TaqMan Master Mix, 5x	4.0	60	68.0	1x
Primers and probe mix, 25x	0.8	12	13.6	1x
Nuclease-free PCR grade water	10.2	153	173.4	–
Sample (to be added at step 4)	5.0	5 each	5.0 each	–
Total volume	20.0	20 each	20.0 each	–

3. **Dispense 15 µl of the qPCR pre-mix per capillary.**
4. **Add 5 µl of the RT product (cDNA, 100 ng RNA equivalent) obtained in the reverse transcription (see "Protocol: Recommended standardized EAC reverse transcription", page 14) in the corresponding tube (total volume 20 µl).**
5. **Mix gently, by pipetting up and down.**
6. **Place the capillaries in the adapters provided with the apparatus, and briefly centrifuge (700 x *g*, approximately 10 seconds).**
7. **Load the capillaries into the thermal cycler according to the manufacturer recommendations.**
8. **Program the LightCycler 1.2 or 2.0 Instrument with the thermal cycling program as indicated in Table 13.**

Table 13. Temperature profile

Mode of analysis	Quantification
Hold	Temperature: 95°C Time: 10 minutes Ramp: 20
Cycling	50 times 95°C for 10 seconds; ramp: 20 60°C for 1 minute; ramp: 20; with acquisition of FAM fluorescence: Single
Hold 2	45°C for 1 minute; ramp: 20

9. **For the LightCycler 1.2, follow step 9a. For the LightCycler 2.0, follow step 9b.**

9a. LightCycler 1.2: The F1/F2 and “2nd derivative analysis” mode is recommended. Start the thermal cycling program, as indicated in Table 13.

9b. LightCycler 2.0: We recommend using Automated (F’’max) analysis on LightCycler 2.0 Software version 4.0 to obtain reproducible results. Start the thermal cycling program, as indicated in Table 13.

Protocol: qPCR on the SmartCycler instrument

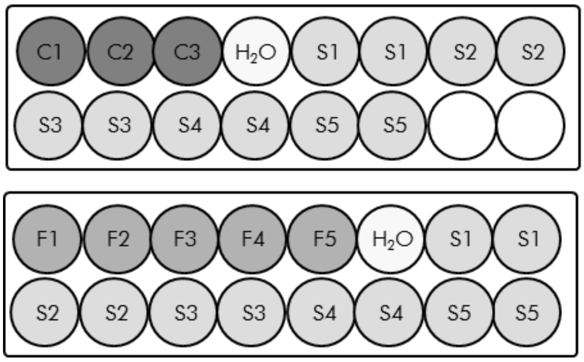
Using this instrument, we recommend measuring samples in duplicate and controls only once, as indicated in Table 14.

Table 14. Number of reactions for the SmartCycler instrument

Samples	Reactions
With the ABL primers and probe mix (PPC-ABL)	
n cDNA samples	n x 2 reactions
ABL standard	1 x 3 reactions (3 standard dilutions, each one tested once)
Water control	1 reaction
With the BCR-ABL Mbcr primers and probe mix (PPF-Mbcr)	
n cDNA samples	n x 2 reactions
Mbcr standard	1 x 5 reactions (5 standard dilutions, each one tested once)
Water control	1 reaction

Sample processing on the SmartCycler instrument

We recommend testing at least 5 cDNA samples in the same experiment to optimize the use of the standards and primers and probe mixes. The two-block scheme in Figure 7 shows an example.



All the assays on this first block are performed with PPC-ABL.

All the assays on this second block are performed with PPF-Mbcr.

Figure 7. Suggested plate setup for one experiment. S: cDNA sample; **F1–5**: BCR-ABL Mbcr standards; **C1–3**: ABL standards; **H_2O**: water control.

qPCR on the SmartCycler instrument

Note: Perform all steps on ice.

Procedure

1. **Thaw all necessary components and place them on ice.**
2. **Prepare the following qPCR mix according to the number of samples being processed.**

   All concentrations are for the final volume of the reaction.

   Table 15 describes the pipetting scheme for the preparation of one reagent mix, calculated to achieve a final reaction volume of 25 µl. A pre-mix can be prepared, according to the number of reactions, using the same primer and probe mix (either PPC-ABL or PPF-Mbcr). Extra volumes are included to compensate for pipetting error.

Table 15. Preparation of qPCR mix

Component	1 reaction (µl)	ABL: 14+1 reactions (µl)	BCR-ABL Mbcr: 16+1 reactions (µl)	Final concentration
TaqMan Universal PCR Master Mix, 2x	12.5	187.5	212.5	1x
Primers and probe mix, 25x	1	15	17	1x
Nuclease-free PCR grade water	6.5	97.5	110.5	–
Sample (to be added at step 4)	5	5 each	5 each	–
Total volume	25	25 each	25 each	–

3. **Dispense 20 µl of the qPCR pre-mix per well.**
4. **Add 5 µl of the RT product (cDNA, 100 ng RNA equivalent) obtained in the reverse transcription (see "Protocol: Recommended standardized EAC reverse transcription", page 14) in the corresponding well (total volume 25 µl).**

5. **Mix gently, by pipetting up and down.**
6. **Load the samples into the thermal cycler according to the manufacturer recommendations.**
7. **Program the SmartCycler instrument with the thermal cycling program as indicated in Table 16.**

Table 16. Temperature profile

Hold	Temperature: 50°C Time: 2 minutes
Hold 2	Temperature: 95°C Time: 10 minutes
Cycling	50 times 95°C for 15 seconds 60°C for 1 minute with acquisition: Single

8. **We recommend a threshold set at 30. Start the thermal cycling program, as indicated in Table 16.**

Interpretation of Results

Data analysis principle

Using TaqMan technology, the number of PCR cycles necessary to detect a signal above the threshold is called the threshold cycle (C_T) and is directly proportional to the amount of target present at the beginning of the reaction.

Using standards with a known number of molecules, one can establish a standard curve and determine the precise amount of target present in the test sample. The *ipsogen* standard curves are plasmid-based; we use 3 plasmid standard dilutions for the CG, and 5 standard dilutions for the FG, in order to ensure accurate standard curves. Figures 8 and 9 show an example of TaqMan amplification curves obtained with the *ipsogen* BCR-ABL Mbcr Kit.

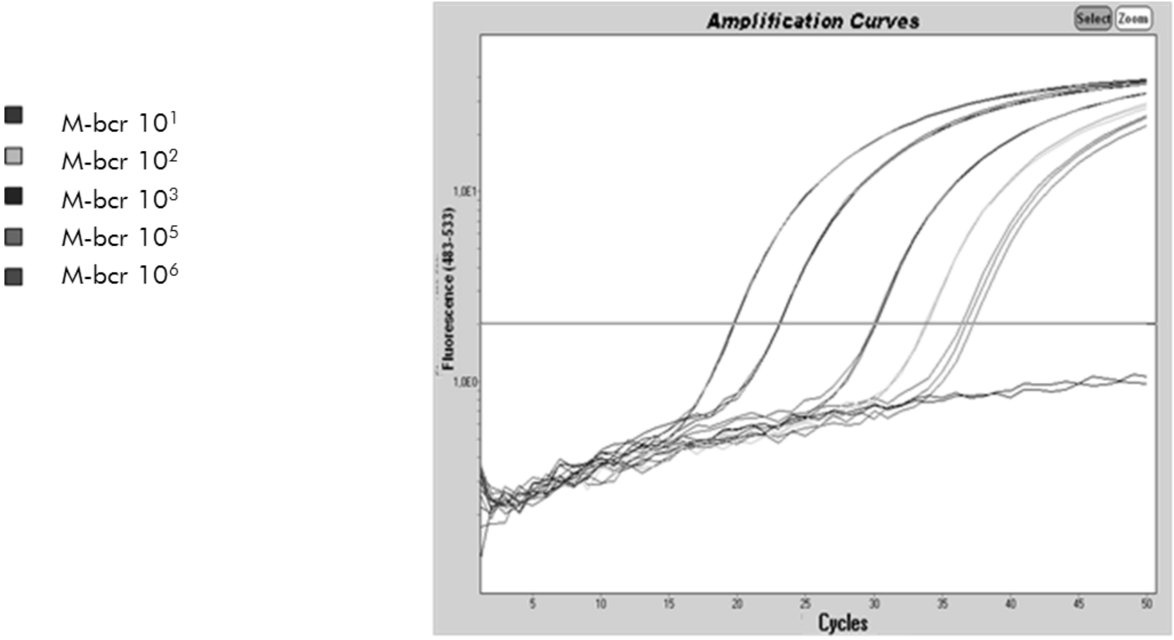


Figure 8. Detection of BCR-ABL Mbcr standards (F1–F5). 10^1, 10^2, 10^3, 10^5, 10^6 copies/5 µl.

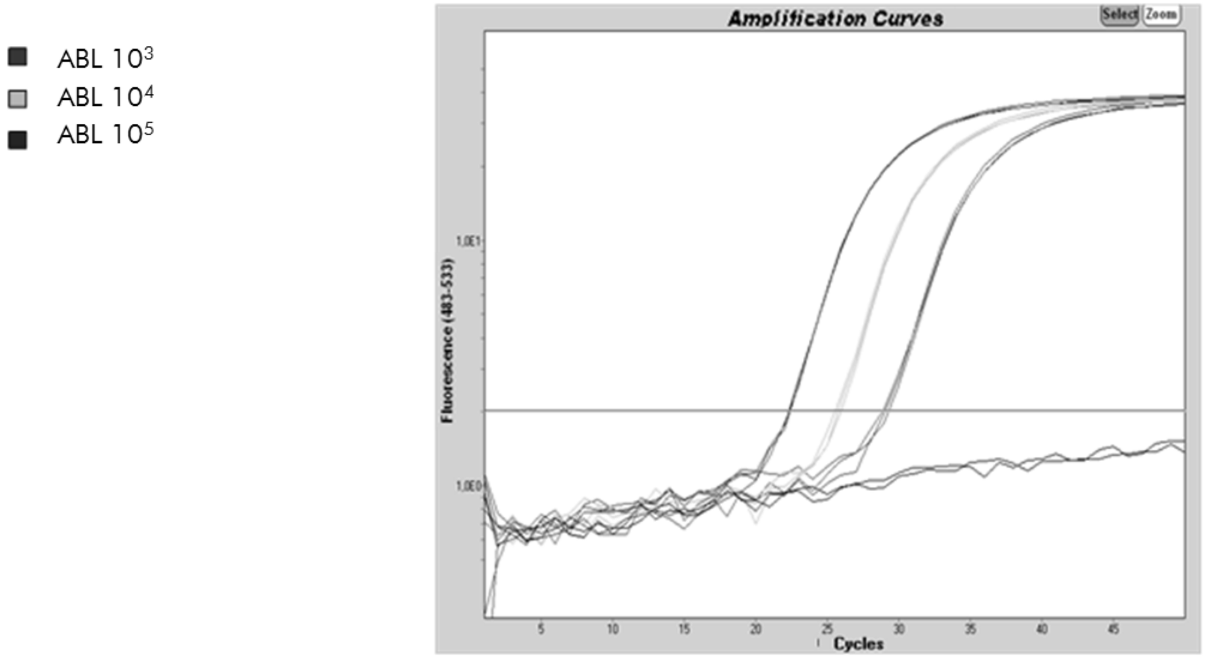


Figure 9. Detection of ABL standards (C1, C2, C3). 10^3, 10^4, and 10^5 copies/5 µl.

Results

Standard curve and quality criteria

Raw data can be pasted into an Excel® file for analysis.

For each gene (ABL and BCR-ABL), raw C_T values obtained from plasmid standard dilutions are plotted according to the log copy number (3, 4, and 5 for C1, C2, and C3; 1, 2, 3, 5, and 6 for F1, F2, F3, F4, and F5). Figure 10 shows an example of the theoretical curve calculated on 5 standard dilutions.

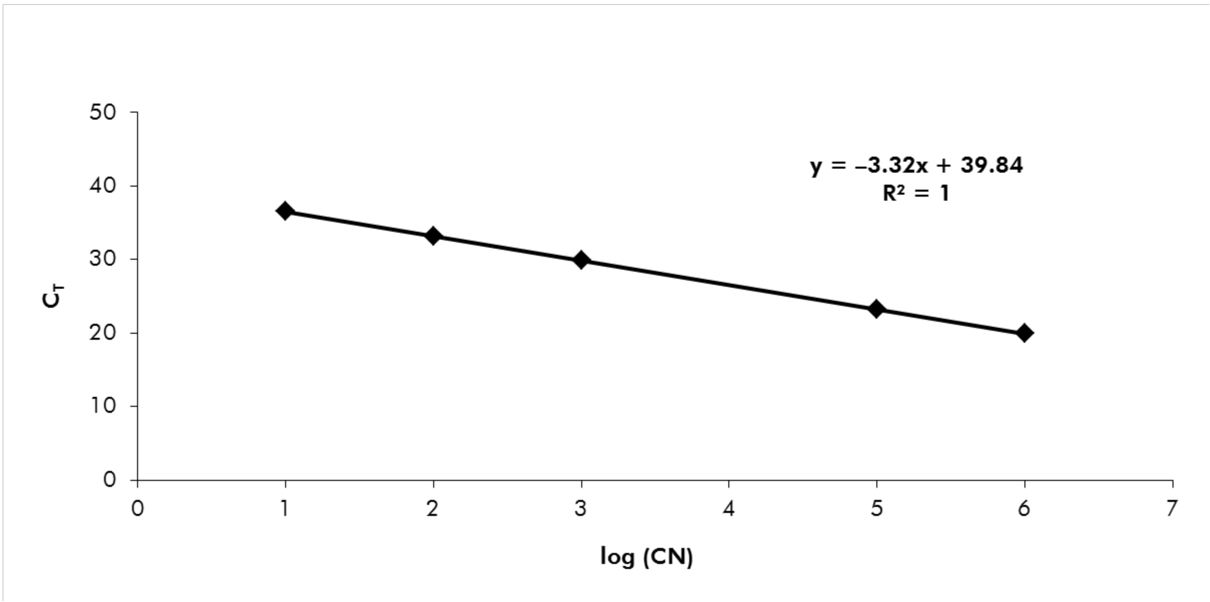


Figure 10. Theoretical curve calculated from the 5 standard dilutions. A linear regression curve ($y = ax + b$) is calculated for each gene (ABL and BCR-ABL), where a is the slope of the line and b is the y-intercept, which is the y-coordinate of the point where the line crosses the y axis. Its equation and coefficient of determination (R^2) are printed on the graph.

As standards are tenfold dilutions, the theoretical slope of the curve is –3.3. A slope between –3.0 and –3.9 is acceptable as long as R^2 is >0.95 (7). However, a value for $R^2 >0.98$ is desirable for precise results (3).

Normalized copy number (NCN)

The ABL standard curve equation should be used to transform raw C_T values (obtained with PPC-ABL) for the unknown samples into ABL copy numbers (ABL_{CN}).

The BCR-ABL standard curve equation should be used to transform raw C_T values (obtained with PPF-Mbcr) for the unknown samples, into BCR-ABL copy numbers (BCR-ABL Mbcr $_{CN}$).

The ratio of these CN values gives the normalized copy number (NCN):

$$\text{NCN} = \frac{\text{BCR-ABL Mbcr}_{\text{CN}}}{\text{ABL}_{\text{CN}}} \times 100$$

MRD value

The minimal residual disease (MRD) value is the ratio between the CG normalized expression of the FG in follow-up $(FG_{CN}/CG_{CN})_{FUP}$ and diagnostic samples $(FG_{CN}/CG_{CN})_{DX}$.

$$\text{MRD value (MRDv)} = \frac{(FG_{CN}/CG_{CN})_{FUP}}{(FG_{CN}/CG_{CN})_{DX}}$$

Sensitivity

The sensitivity (SENSv) is calculated according to the relative expression of the FG at diagnosis $(FG_{CN}/CG_{CN})_{DX}$ and CG expression ($CG_{CN,FUP}$) in the follow-up sample.

$$\text{Sensitivity (SENSv)} = \frac{CG_{CN,DX}}{CG_{CN,FUP} \times FG_{CN,DX}}$$

Quality control on ABL values

Poor quality of the RNA or problems during the qPCR steps result in low ABL_{CN}. We recommend discarding results from samples giving ABL_{CN} <4246.2 (lower value of the 95% CI from CML patient samples in the EAC study, reference 8).

Reproducibility between replicates

The variation in C_T values between replicates should be <2, corresponding to a fourfold change in copy number values.

Variation in C_T values between replicates is generally <1.5 if the mean C_T value of the replicates is <36 (7).

Note: Each user should measure their own reproducibility in their laboratory.

Water controls

Negative controls should give zero CN.

A positive water control results from a cross-contamination. See "Troubleshooting guide", below, to find a solution.

Troubleshooting guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocol in this handbook or sample and assay technologies (for contact information, see "Contact Information", page 47).

	Comments and suggestions
Negative result for the control gene (ABL) and BCR-ABL Mbcr in all the samples — standard okay	
a) Poor RNA quality	Always check the RNA quality and concentration before starting. Run a cell line RNA positive control (*ipsogen* BCR-ABL1 Mbcr Controls Kit, cat. no. 670191) in parallel.
b) Failure of reverse transcription step	Always check the RNA quality and concentration before starting. Run a cell line RNA positive control (*ipsogen* BCR-ABL1 Mbcr Controls Kit, cat. no. 670191) in parallel.
Negative result for the control gene (ABL) in the samples — standard okay	
a) Poor RNA quality	Always check the RNA quality and concentration before starting. Run a cell line RNA positive control (*ipsogen* BCR-ABL1 Mbcr Controls Kit, cat. no. 670191) in parallel.
b) Failure of reverse transcription step	Always check the RNA quality and concentration before starting. Run a cell line RNA positive control (*ipsogen* BCR-ABL1 Mbcr Controls Kit, cat. no. 670191) in parallel.
Standard signal negative	
a) Pipetting error	Check pipetting scheme and the setup of the reaction. Repeat the PCR run.
b) Inappropriate storage of kit components	Store the *ipsogen* BCR-ABL1 Mbcr Kit at –15 to –30°C and keep primers and probe mixes (PPC and PPF) protected from light. See "Reagent Storage and Handling", page 13. Avoid repeated freezing and thawing. Aliquot reagents for storage.

	Comments and suggestions
Negative controls are positive	
Cross-contamination	Replace all critical reagents. Repeat the experiment with new aliquots of all reagents. Always handle samples, kit components, and consumables in accordance with commonly accepted practices to prevent carry-over contamination.
No signal, even in standard controls	
a) Pipetting error or omitted reagents	Check pipetting scheme and the setup of the reaction. Repeat the PCR run.
b) Inhibitory effects of the sample material, caused by insufficient purification	Repeat the RNA preparation.
c) LightCycler: Incorrect detection channel chosen	Set Channel Setting to F1/F2 or 530 nm/640 nm.
d) LightCycler: No data acquisition programmed	Check the cycle programs. Select acquisition mode “single” at the end of each annealing segment of the PCR program.
Absent or low signal in samples but standard controls okay	
a) Poor RNA quality or low concentration	Always check the RNA quality and concentration before starting. Run a cell line RNA positive control (*ipsogen* BCR-ABL1 Mbcr Controls Kit, cat. no. 670191) in parallel.
b) Failure of reverse transcription step	Always check the RNA quality and concentration before starting. Run a cell line RNA positive control (*ipsogen* BCR-ABL1 Mbcr Controls Kit, cat. no. 670191) in parallel.

	Comments and suggestions
Fluorescence intensity too low	
a) Inappropriate storage of kit components	Store the *ipsogen* BCR-ABL1 Mbcr Kit at –15 to –30°C and keep primers and probe mixes (PPC and PPF) protected from light. See “Reagent Storage and Handling”, page 13. Avoid repeated freezing and thawing. Aliquot reagents for storage.
b) Very low initial amount of target RNA	Increase the amount of sample RNA. **Note**: Depending of the chosen method of RNA preparation, inhibitory effects may occur.
LightCycler: Fluorescence intensity varies	
a) Pipetting error	Variability caused by so-called “pipetting error” can be reduced by analyzing data in the F1/F2 or 530 nm/640 nm mode.
b) Insufficient centrifugation of the capillaries	The prepared PCR mix may still be in the upper vessel of the capillary, or an air bubble could be trapped in the capillary tip. Always centrifuge capillaries loaded with the reaction mix as described in the specific operating manual of the apparatus.
c) Outer surface of the capillary tip dirty	Always wear gloves when handling the capillaries.
LightCycler: Error of the standard curve	
Pipetting error	Variability caused by so-called “pipetting error” can be reduced by analyzing data in the F1/F2 or 530 nm/640 nm mode.

Quality Control

Quality control of the complete kit has been performed on a LightCycler 480 Instrument. This kit is manufactured according to ISO 13485:2003 standard. Certificates of analysis are available on request at www.qiagen.com/support/.

Limitations

The users must be trained and familiar with this technology prior to the use of this device.

Any diagnostic results generated must be interpreted in conjunction with other clinical or laboratory findings. It is the user's responsibility to validate system performance for any procedures used in their laboratory which are not covered by the QIAGEN performance studies.

Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.

Note: The kit has been designed according to the "Europe Against Cancer" (EAC) studies (8), and is compliant with the updated international recommendations (3, 5). It should be used following the instructions given in this manual, in combination with validated reagents and instruments (see "Materials Required but Not Provided", page 11). Any off-label use of this product and/or modification of the components will void QIAGEN's liability.

Performance Characteristics

Nonclinical studies

Materials and methods

Performance evaluation was performed on an ABI PRISM 7700 SDS, in combination with reagents listed in "Materials Required but Not Provided", page 11. Equivalence studies validated its use on the following instruments: ABI PRISM 7000 and 7900HT SDS, LightCycler 1.2 and 480 Instruments, Rotor-Gene 3000, and SmartCycler instrument (9).

Nonclinical studies were conducted to establish the analytical performance of the *ipsogen* BCR-ABL1 Mbcr Kit. These nonclinical laboratory studies were performed on total RNA from K562 cell line diluted in a constant final amount of MV4-11 cell line total RNA.

To determine the repeatability of the assay, 5 different concentrations of K562 total RNA (5 ng, 500 pg, 50 pg, 5 pg, and 0.5 pg) diluted in MV4-11 total RNA, in a constant final total amount of 200 ng, were analyzed in 5 replicates per run and in 4 different runs (Figure 11).

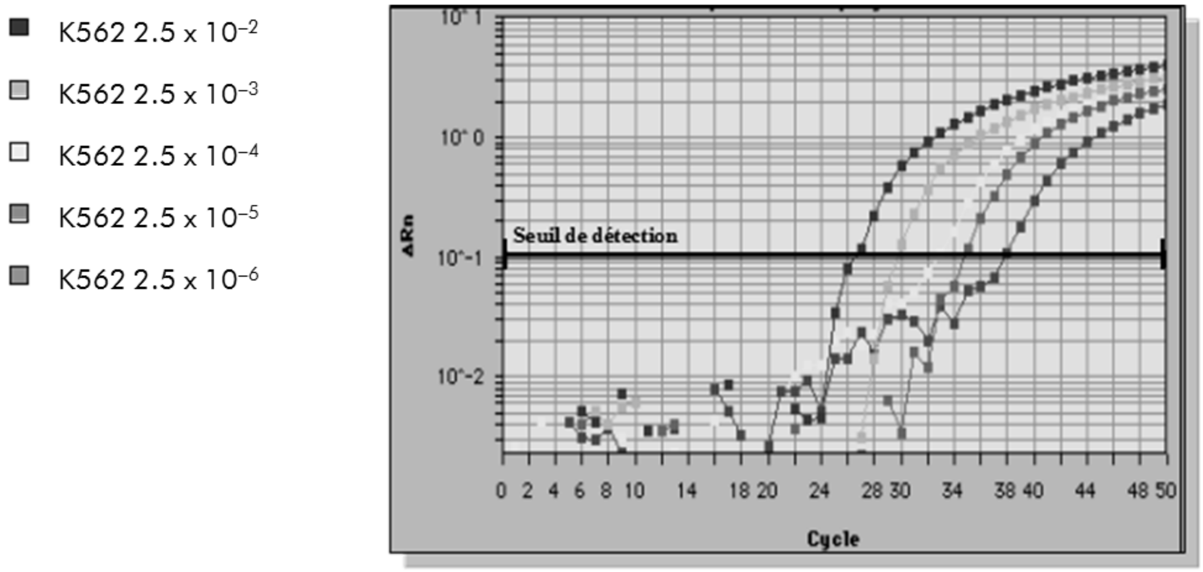


Figure 11. Amplification plots of 2.5 x 10^{-2} (5 ng), 2.5 x 10^{-4} (0.05 ng), 2.5 x 10^{-5} (0.005 ng), and 2.5 x 10^{-6} (0.0005 ng) dilutions of K562 total RNA in MV4-11 negative total RNA.

Analytical data

Tables 17–20 show the inter-assay analyses with the mean threshold cycle (C_T), standard deviation (SD), number of samples (n), coefficient of variation (CV), mean copy number (CN), and mean normalized copy number (NCN).

Table 17. Inter-assay analysis — cell lines BCR-ABL Mbcr and ABL

Cell line	Dilution	Mean C_T	SD	n	CV (%)
BCR-ABL Mbcr	2.5 x 10^{-2} (5 ng/200 ng)	26.18	0.40	20	1.54
	2.5 x 10^{-3} (0.5 ng/200 ng)	29.32	0.53	19	1.82
	2.5 x 10^{-4} (0.05 ng/200 ng)	32.62	0.62	20	1.91
ABL	–	23.59	0.20	95	0.83

Table 18. Inter-assay analysis — plasmids

Gene	Plasmid	Mean C_T	SD	n	CV (%)
BCR-ABL Mbcr	F1 (10^1 copies)	34.47	1.25	8	3.64
	F2 (10^2 copies)	31.48	0.54	8	1.71
	F3 (10^3 copies)	28.17	1.11	7	3.95
	F4 (10^5 copies)	21.20	0.65	8	3.06
	F5 (10^6 copies)	18.22	0.09	6	0.49
ABL	C1 (10^3 copies)	28.47	0.34	8	1.18
	C2 (10^4 copies)	25.25	0.31	8	1.22
	C3 (10^5 copies)	21.92	0.70	8	3.19

Table 19. Inter-assay analysis — cell lines BCR-ABL Mbcr and ABL (mean CN)

Cell line	Dilution	Mean CN	SD	n	CV (%)
BCR-ABL Mbcr	2.5×10^{-2} (5 ng/200 ng)	4134.27	2512.40	20	60.77
	2.5×10^{-3} (0.5 ng/200 ng)	512.8	479.51	19	93.51
	2.5×10^{-4} (0.05 ng/200 ng)	42.94	22.05	20	51.36
ABL	–	33,831.51	13,637.7	94	40.31

Table 20. Inter-assay analysis — cell line BCR-ABL Mbcr (mean NCN)

Cell line	Dilution	Mean NCN*	SD	n	CV (%)
BCR-ABL Mbcr	2.5×10^{-2} (5 ng/200 ng)	12.6338	532.79	20	42.17
	2.5×10^{-3} (0.5 ng/200 ng)	1.1605	94.69	19	81.61
	2.5×10^{-4} (0.05 ng/200 ng)	0.1782	10.73	20	60.23

* For these study results only, the NCN is given as $\frac{\text{Mbcr}_{\text{CN}}}{\text{ABL}_{\text{CN}}} \times 100$.

Clinical studies

Performance evaluation was performed on an ABI PRISM 7700 SDS, in combination with reagents listed in “Materials Required but Not Provided”, page 11. Equivalence studies validated its use on the following instruments: ABI PRISM 7000 and 7900HT SDS, LightCycler 1.2 and 480 Instruments, Rotor-Gene 3000, and SmartCycler instrument (9).

A group of 26 laboratories, in 10 European countries, organized in a Europe Against Cancer (EAC) concerted action, used plasmids provided by IPSOGEN to establish a standardized protocol for qPCR analysis of the major leukemia-associated fusion genes in the clinical setting. The BCR-ABL p210 transcript was one of the fusion genes (FG) included in the study. We present here a summary of this validation study; full results have been published (8, 10).

Inter-laboratory reproducibility for CG and FG plasmid standards

Eleven laboratories performed an inter-laboratory reproducibility experiment to assess variability in the measurement of CG and FG plasmid standard dilutions. Dilutions were performed in duplicate at each facility. Table 21 reports the mean, standard deviation, and CV (%) for each dilution.

Table 21. Inter-laboratory reproducibility for CG and FG plasmid standards

Gene	Dilution	Mean	C_T SD	CV (%)
ABL control gene	C1	29.59	1.34	4.54
	C2	26.33	1.02	3.90
	C3	22.75	1.59	6.97
BCR-ABL p210 fusion gene	F1	41.11	2.26	5.50
	F2	37.43	1.51	4.04
	F3	33.76	1.28	3.81
	F4	26.50	1.03	3.90
	F5	22.98	0.97	4.21

Expression values of the BCR-ABL Mbcr FG transcript

Tables 22 and 23 show the expression values of the BCR-ABL Mbcr FG transcript and ABL CG, for K562 cell line, CML and ALL patients at diagnosis, and normal patients.

Table 22. Expression values of the BCR-ABL Mbcr FG transcript and ABL CG — C_T values

	C_T values (95% range)	
	BCR-ABL Mbcr	**ABL**
K562 cell line	20.5	20.7
CML patient samples		
BM (n = 15)	25.1 (21.5–27.0)	25.2 (20.7–26.8)
PB (n = 14)	23.1 (21.9–25.8)	23.7 (22.6–26.7)
ALL patient samples		
BM and PB (n = 17)	24.1 (21.5–29.9)	24.0 (21.6–26.4)
Negative patient samples		
BM (n = 26)	–	25.35 (24.68–26.02)
PB (n = 74)	–	25.15 (24.83–25.48)

Table 23. Expression values of the BCR-ABL Mbcr FG transcript and ABL CG — CN and ratio values

	CN values (95% range)		Ratio values (95% range)*
	BCR-ABL Mbcr	**ABL**	**CN BCR-ABL Mbcr/CN ABL**
CML patient samples			
BM (n = 15)	8710 (2089–112,202)	10,115.8 (4786.3–37,153.52)	0.86 (0.44–3.02)
PB (n = 14)	17,783 (2042–112,202)	15,237 (4246.2–25,568.3)	1.17 (0.48–4.41)
Negative patient samples			
BM (n = 26)	–	19,201 (12,922–25,480)	–
PB (n = 74)	–	21,136 (17,834–24,437)	–

* Results are expressed as simple BCR-ABL/ABL ratios.

ABL C_T values did not differ significantly between normal and leukemia samples, nor between samples types (PB or BM) or leukemia samples (ALL, AML, CML).

False positive and false negative rates

False negative and false positive rates were computed using the following controls.

- Positive controls: K562 cells, a cell line well known for its positivity for BCR-ABL p210 fusion gene; patients samples already assessed for p210 positivity
- Negative controls: Negative RNA samples, no amplification controls (NAC) made of *E. coli* RNA instead of human RNA to check for PCR contamination, and no template controls (NTC), which contained water instead of human RNA

Amplification on RNA samples of the FG was done in triplicate and in duplicate for the CG.

A false-negative sample was defined as a positive RNA sample with less than 50% of positive wells (0/2, 0/3, or 1/3).

A false-positive sample was defined as a negative sample with at least 50% of positive wells (1/2, 2/3, or 3/3).

Table 24 shows the number and percentage of false negative and false positive samples.

Table 24. False negative and false positive samples

False negativity		**False positivity**	
10^{-3}	**10^{-4}**	**FG negative control**	**NAC/NTC**
0% (0/33)	6.1% (2/33)	10.9% (6/55)	4.1% (14/340)

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

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9. Silvy, M., Mancini, J., Thirion, X., Sigaux, F., and Gabert, J. (2005) Evaluation of real-time quantitative PCR machines for the monitoring of fusion gene transcripts using the Europe against cancer protocol. Leukemia **19**, 305.
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Symbols

The following symbols may appear on the packaging and labeling:

Σ <N>	Contains reagents sufficient for <N> reactions
⌛	Use by
IVD	In vitro diagnostic medical device
REF	Catalog number
LOT	Lot number
MAT	Material number
GTIN	Global Trade Item Number
🌡	Temperature limitation
🏭	Manufacturer
ℹ	Consult instructions for use

Contact Information

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support, call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Ordering Information

Product	Contents	Cat. no.
ipsogen BCR-ABL1 Mbcr Kit (24)	For 24 reactions: ABL Control Gene Standards, BCR-ABL Mbcr Fusion Gene Standards, Primer and Probe Mix ABL, Primer and Probe Mix BCR-ABL Mbcr Fusion Gene	670123
Rotor-Gene Q MDx — for IVD-validated real-time PCR analysis in clinical applications		
Rotor-Gene Q MDx 5plex HRM Platform	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9002032
Rotor-Gene Q MDx 5plex HRM System	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9002033
***ipsogen* BCR-ABL1 Mbcr Controls Kit — for qualitative validation of RNA extraction and reverse transcription of the BCR-ABL Mbcr fusion gene**		
ipsogen BCR-ABL1 Mbcr Controls Kit	Cell lines with negative, high, and low positive expression of the BCR-ABL Mbcr fusion gene	670191

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