March 2015

ipsogen® FusionQ*uant*® Handbook

Σ 24 (various)

Σ 52 (catalog no. 670115)

For research use only. Not for use in diagnostic procedures.

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

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672213, 672313, 675013, 675113, 676013, 670115

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANY

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Intended Use

The *ipsogen* FusionQ*uant* kits are intended for research use only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Principle of the Procedure

Some chromosomal translocations result in the creation of fusion gene transcripts, which can be tested with real-time quantitative PCR (qPCR). This technology combines DNA amplification with detection of the products in a single tube.

In the *ipsogen* FusionQ*uant* kits, an endogenous control transcript (ABL, GUS, or BCR) is amplified from the sample together with the fusion transcript of interest. Standard curves of known amounts of both the endogenous control and the fusion cDNA allow the calculation of the ratio of specific fusion transcript signal to endogenous control gene signal in each sample. Specific primers and probe mixes and standard serial dilutions of control and fusion DNA are provided for the quantification of the control and fusion genes.

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.

These assays exploit the qPCR double-dye oligonucleotide hydrolysis principle. During PCR, forward and reverse primers hybridize to a specific sequence (see example for BCR-ABL1 Mbcr in Figure 1). 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.

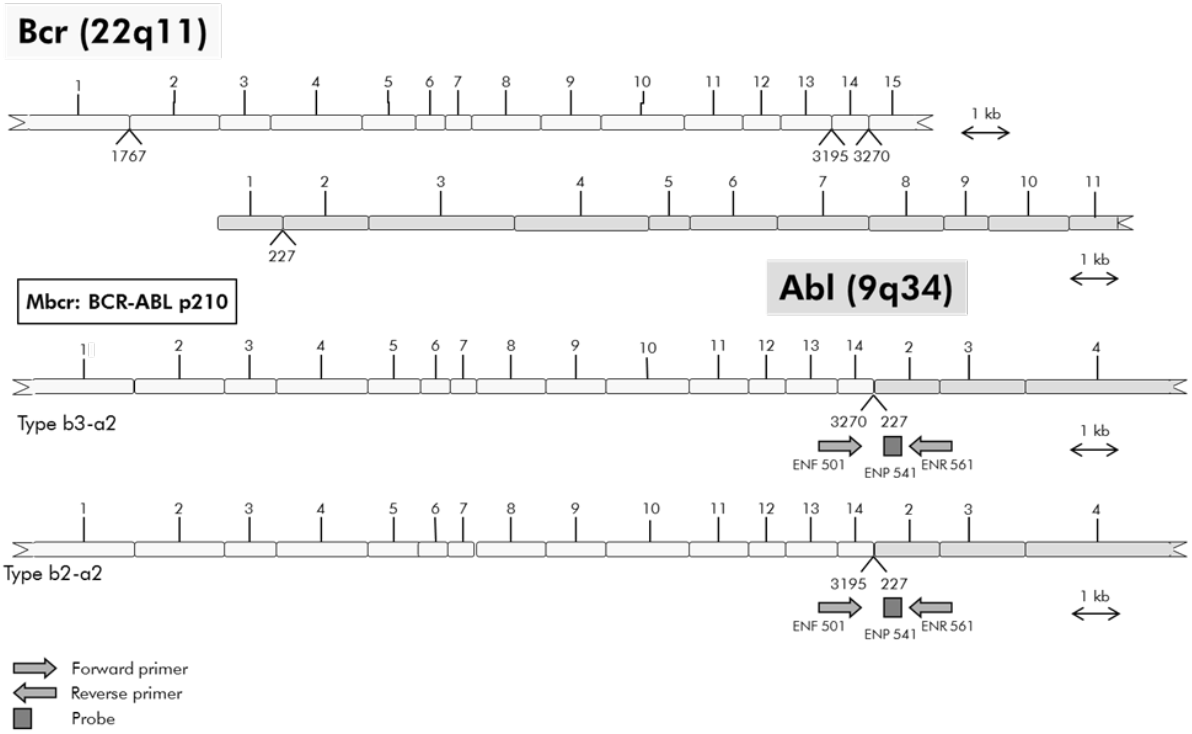

Figure 1. Schematic diagram of the BCR-ABL Mbcr fusion gene transcript covered by the qPCR primers and probe set: ENF501–ENP541–ENR561. The number under the primers and probe refers to their nucleotide position in the normal gene transcript.

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 2). 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.

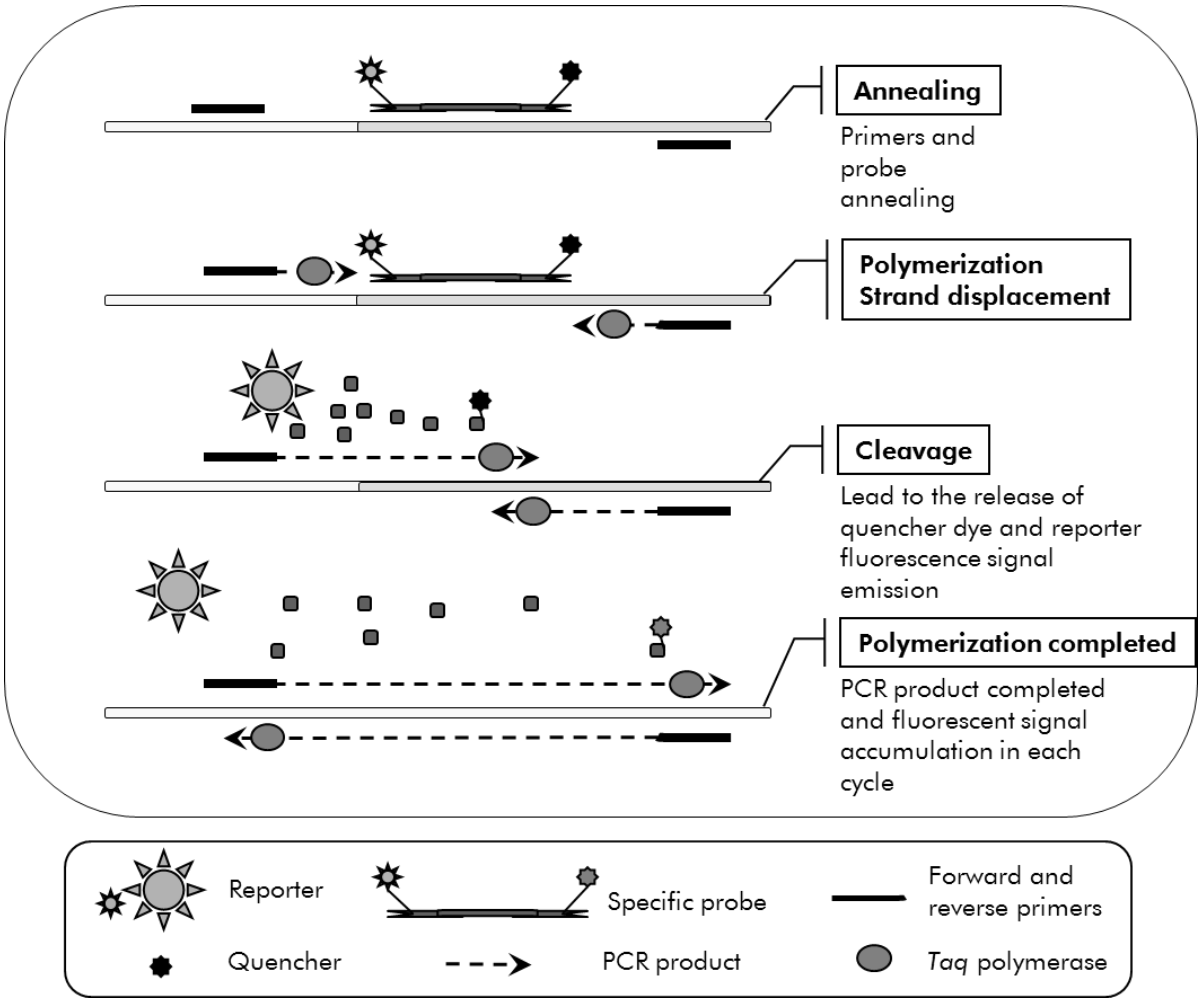

Figure 2. Reaction principle. Total RNA is reverse-transcribed, and the generated cDNA is amplified by PCR using a pair of specific primers and a specific internal double-dye probe (FAM™–TAMRA™). The probe binds to the amplicon during each annealing step of the PCR. When the *Taq* DNA polymerase extends 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.		**670013**
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

* 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.

Kit contents

***ipsogen* BCR-ABL1 Mbcr Kit**		**(24)**
Catalog no.		**670113**
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

* 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.

Kit contents

***ipsogen* BCR-ABL1 Mbcr Kit**		**(52)**
Catalog no.		**670115**
Number of reactions		**52**
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	2 x 90 µl
Primers and Probe Mix BCR-ABL Mbcr Fusion Gene†	PPF-Mbcr 25x	2 x 110 µl

* 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.

Kit contents

***ipsogen* BCR-ABL1 mbcr (BCR) Kit**		**(24)**
Catalog no.		**670213**
Number of reactions		**24**
BCR Control Gene Standard Dilution (10^3 copies/5 µl)	C1-BCR	50 µl
BCR Control Gene Standard Dilution (10^4 copies/5 µl)	C2-BCR	50 µl
BCR Control Gene Standard Dilution (10^5 copies/5 µl)	C3-BCR	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 BCR*	PPC-BCR 25x	90 µl
Primers and Probe Mix BCR-ABL mbcr Fusion Gene†	PPF-mbcr 25x	110 µl

* Mix of specific reverse and forward primers for the BCR 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.

Kit contents

***ipsogen* BCR-ABL1 Mbcr (BCR) Kit**		**(24)**
Catalog no.		**670313**
Number of reactions		**24**
BCR Control Gene Standard Dilution (10^3 copies/5 µl)	C1-BCR	50 µl
BCR Control Gene Standard Dilution (10^4 copies/5 µl)	C2-BCR	50 µl
BCR Control Gene Standard Dilution (10^5 copies/5 µl)	C3-BCR	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 BCR*	PPC-BCR 25x	90 µl
Primers and Probe Mix BCR-ABL Mbcr Fusion Gene†	PPF-Mbcr 25x	110 µl

* Mix of specific reverse and forward primers for the BCR 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.

Kit contents

***ipsogen* BCR-ABL1 mbcr (GUS) Kit**		**(24)**
Catalog no.		**670413**
Number of reactions		**24**
GUS Control Gene Standard Dilution (10^3 copies/5 µl)	C1-GUS	50 µl
GUS Control Gene Standard Dilution (10^4 copies/5 µl)	C2-GUS	50 µl
GUS Control Gene Standard Dilution (10^5 copies/5 µl)	C3-GUS	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 GUS*	PPC-GUS 25x	90 µl
Primers and Probe Mix BCR-ABL mbcr Fusion Gene†	PPF-mbcr 25x	110 µl

* Mix of specific reverse and forward primers for the GUS 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.

Kit contents

***ipsogen* BCR-ABL1 Mbcr (GUS) Kit**		**(24)**
Catalog no.		**670513**
Number of reactions		**24**
GUS Control Gene Standard Dilution (10^3 copies/5 µl)	C1-GUS	50 µl
GUS Control Gene Standard Dilution (10^4 copies/5 µl)	C2-GUS	50 µl
GUS Control Gene Standard Dilution (10^5 copies/5 µl)	C3-GUS	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 GUS*	PPC-GUS 25x	90 µl
Primers and Probe Mix BCR-ABL Mbcr Fusion Gene†	PPF-Mbcr 25x	110 µl

* Mix of specific reverse and forward primers for the GUS 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.

Kit contents

***ipsogen* PML-RARA bcr1 Kit**		**(24)**
Catalog no.		**672113**
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
PML-RARA bcr1 Fusion Gene Standard Dilution (10^1 copies/5 µl)	F1-PML-RARA bcr1	50 µl
PML-RARA bcr1 Fusion Gene Standard Dilution (10^2 copies/5 µl)	F2-PML-RARA bcr1	50 µl
PML-RARA bcr1 Fusion Gene Standard Dilution (10^3 copies/5 µl)	F3-PML-RARA bcr1	50 µl
PML-RARA bcr1 Fusion Gene Standard Dilution (10^5 copies/5 µl)	F4-PML-RARA bcr1	50 µl
PML-RARA bcr1 Fusion Gene Standard Dilution (10^6 copies/5 µl)	F5-PML-RARA bcr1	50 µl
Primers and Probe Mix ABL*	PPC-ABL 25x	90 µl
Primers and Probe Mix PML-RARA bcr1 Fusion Gene†	PPF-bcr1 25x	110 µl

* 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 PML-RARA bcr1 fusion gene plus a specific FAM–TAMRA probe.

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

Kit contents

***ipsogen* PML-RARA bcr2 Kit**		**(24)**
Catalog no.		**672213**
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
PML-RARA bcr2 Fusion Gene Standard Dilution (10^1 copies/5 µl)	F1-PML-RARA bcr2	50 µl
PML-RARA bcr2 Fusion Gene Standard Dilution (10^2 copies/5 µl)	F2-PML-RARA bcr2	50 µl
PML-RARA bcr2 Fusion Gene Standard Dilution (10^3 copies/5 µl)	F3-PML-RARA bcr2	50 µl
PML-RARA bcr2 Fusion Gene Standard Dilution (10^5 copies/5 µl)	F4-PML-RARA bcr2	50 µl
PML-RARA bcr2 Fusion Gene Standard Dilution (10^6 copies/5 µl)	F5-PML-RARA bcr2	50 µl
Primers and Probe Mix ABL*	PPC-ABL 25x	90 µl
Primers and Probe Mix PML-RARA bcr2 Fusion Gene†	PPF-bcr2 25x	110 µl

* 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 PML-RARA bcr2 fusion gene plus a specific FAM–TAMRA probe.

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

Kit contents

***ipsogen* PML-RARA bcr3 Kit**		**(24)**
Catalog no.		**672313**
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
PML-RARA bcr3 Fusion Gene Standard Dilution (10^1 copies/5 µl)	F1-PML-RARA bcr3	50 µl
PML-RARA bcr3 Fusion Gene Standard Dilution (10^2 copies/5 µl)	F2-PML-RARA bcr3	50 µl
PML-RARA bcr3 Fusion Gene Standard Dilution (10^3 copies/5 µl)	F3-PML-RARA bcr3	50 µl
PML-RARA bcr3 Fusion Gene Standard Dilution (10^5 copies/5 µl)	F4-PML-RARA bcr3	50 µl
PML-RARA bcr3 Fusion Gene Standard Dilution (10^6 copies/5 µl)	F5-PML-RARA bcr3	50 µl
Primers and Probe Mix ABL*	PPC-ABL 25x	90 µl
Primers and Probe Mix PML-RARA bcr3 Fusion Gene†	PPF-bcr3 25x	110 µl

* 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 PML-RARA bcr3 fusion gene plus a specific FAM–TAMRA probe.

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

Kit contents

***ipsogen* RUNX1-RUNX1T1 Kit**		**(24)**
Catalog no.		**675013**
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
RUNX1-RUNX1T1 Fusion Gene Standard Dilution (10^1 copies/5 µl)	F1-RUNX1-RUNX1T1	50 µl
RUNX1-RUNX1T1 Fusion Gene Standard Dilution (10^2 copies/5 µl)	F2-RUNX1-RUNX1T1	50 µl
RUNX1-RUNX1T1 Fusion Gene Standard Dilution (10^3 copies/5 µl)	F3-RUNX1-RUNX1T1	50 µl
RUNX1-RUNX1T1 Fusion Gene Standard Dilution (10^5 copies/5 µl)	F4-RUNX1-RUNX1T1	50 µl
RUNX1-RUNX1T1 Fusion Gene Standard Dilution (10^6 copies/5 µl)	F5-RUNX1-RUNX1T1	50 µl
Primers and Probe Mix ABL*	PPC-ABL 25x	90 µl
Primers and Probe Mix RUNX1-RUNX1T1 Fusion Gene†	PPF-RUNX1T1 25x	110 µl

* 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 RUNX1-RUNX1T1 fusion gene plus a specific FAM–TAMRA probe.

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

Kit contents

***ipsogen* ETV6-RUNX1 Kit**	**(24)**	
Catalog no.	**675113**	
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
ETV6-RUNX1 Fusion Gene Standard Dilution (10^1 copies/5 µl)	F1-ETV6-RUNX1	50 µl
ETV6-RUNX1 Fusion Gene Standard Dilution (10^2 copies/5 µl)	F2-ETV6-RUNX1	50 µl
ETV6-RUNX1 Fusion Gene Standard Dilution (10^3 copies/5 µl)	F3-ETV6-RUNX1	50 µl
ETV6-RUNX1 Fusion Gene Standard Dilution (10^5 copies/5 µl)	F4-ETV6-RUNX1	50 µl
ETV6-RUNX1 Fusion Gene Standard Dilution (10^6 copies/5 µl)	F5-ETV6-RUNX1	50 µl
Primers and Probe Mix ABL*	PPC-ABL 25x	90 µl
Primers and Probe Mix ETV6-RUNX1 Fusion Gene†	PPF-RUNX1 25x	110 µl

* 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 ETV-RUNX1 fusion gene plus a specific FAM–TAMRA probe.

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

Kit contents

***ipsogen* CBFB-MYH11A Kit**		**(24)**
Catalog no.		**676013**
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
CBFB-MYH11A Fusion Gene Standard Dilution (10^1 copies/5 µl)	F1-CBFB-MYH11A	50 µl
CBFB-MYH11A Fusion Gene Standard Dilution (10^2 copies/5 µl)	F2-CBFB-MYH11A	50 µl
CBFB-MYH11A Fusion Gene Standard Dilution (10^3 copies/5 µl)	F3-CBFB-MYH11A	50 µl
CBFB-MYH11A Fusion Gene Standard Dilution (10^5 copies/5 µl)	F4-CBFB-MYH11A	50 µl
CBFB-MYH11A Fusion Gene Standard Dilution (10^6 copies/5 µl)	F5-CBFB-MYH11A	50 µl
Primers and Probe Mix ABL*	PPC-ABL 25x	90 µl
Primers and Probe Mix CBFB-MYH11A Fusion Gene†	PPF-MYH11A 25x	110 µl

* 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 CBFB-MYH11A 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 recommended 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 recommended reagent is RNaseOUT™ (Life Technologies, cat. no. 10777-019)
- Set of dNTPs, PCR grade
- Random nonamer
- $MgCl_2$
- Buffer and *Taq* DNA polymerase: The recommended 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 5plex HRM or other Rotor-Gene instrument; LightCycler 1.2, 1.5, 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)
- Spectrophotometer*

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

Complementary reagents

- Control kits consisting of cell lines with negative, high, and low positive expression of fusion genes are available for the qualitative testing of the RNA extraction and the reverse transcription (*ipsogen* BCR-ABL mbcr Controls Kit, cat. no. 670091; *ipsogen* BCR-ABL1 Mbcr Controls Kit, cat. no. 670191; *ipsogen* PML-RARA bcr1 Controls Kit, cat. no. 672091).
- A kit for reverse transcription to obtain cDNA from cell lines is available (*ipsogen* RT Kit, cat. no. 679913). When using this kit, please refer to the specific protocol provided in the handbook.

Warnings and Precautions

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.

These kits are intended for research use. Reagents and instructions supplied in these kits have been tested 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 these kits. For optimal performance of the kits, 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, plasmid, or PCR products) 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.

Sample Storage and Handling

Whole blood samples should be anti-coagulated with potassium EDTA and stored at 2–8°C for no more than 5 days before RNA extraction.

Procedure

Sample RNA preparation

RNA extraction should be performed with a recommended procedure (QIAGEN RNeasy® Mini Kit, cat. no 74104 or RNeasy Midi Kit, cat. no. 75144; or Life Technologies TRIzol®, cat. nos. 15596-026 and 15596-018).

The performance of an assay is dependent on the concentration and quality of input RNA. We therefore recommend qualifying the purified RNA, prior to downstream analysis, by agarose* gel electrophoresis, Agilent® Bioanalyzer®, or spectrophotometry.

Protocol: 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 1).**

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

Table 1. 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 nonamer (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 2).

Table 2. 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 spin (approximately 5 seconds, 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 5plex HRM instruments with 72-tube rotor

Using this instrument, we recommend performing all measurements in duplicate, as indicated in Table 3.

Table 3. Number of reactions for Rotor-Gene Q instruments with 72-tube rotor

Samples	Reactions
With the control gene primers and probe mix (PPC)	
n cDNA samples	n x 2 reactions
Control gene standard	2 x 3 reactions (3 dilutions, each one tested in duplicate)
Water control	2 reactions
With the fusion gene primers and probe mix (PPF)	
n cDNA samples	n x 2 reactions
Fusion gene 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* FusionQ*uant* kit provides enough reagents to perform an 8-sample experiment 3 times using the 72-tube rotor. The *ipsogen* BCR-ABL-Mbcr Kit for 52 reactions (cat. no. 670115) provides enough reagents to perform a 13-sample experiment 4 times using the 72-tube rotor.

The rotor scheme in Figure 3 shows an example of an 8-sample experiment.

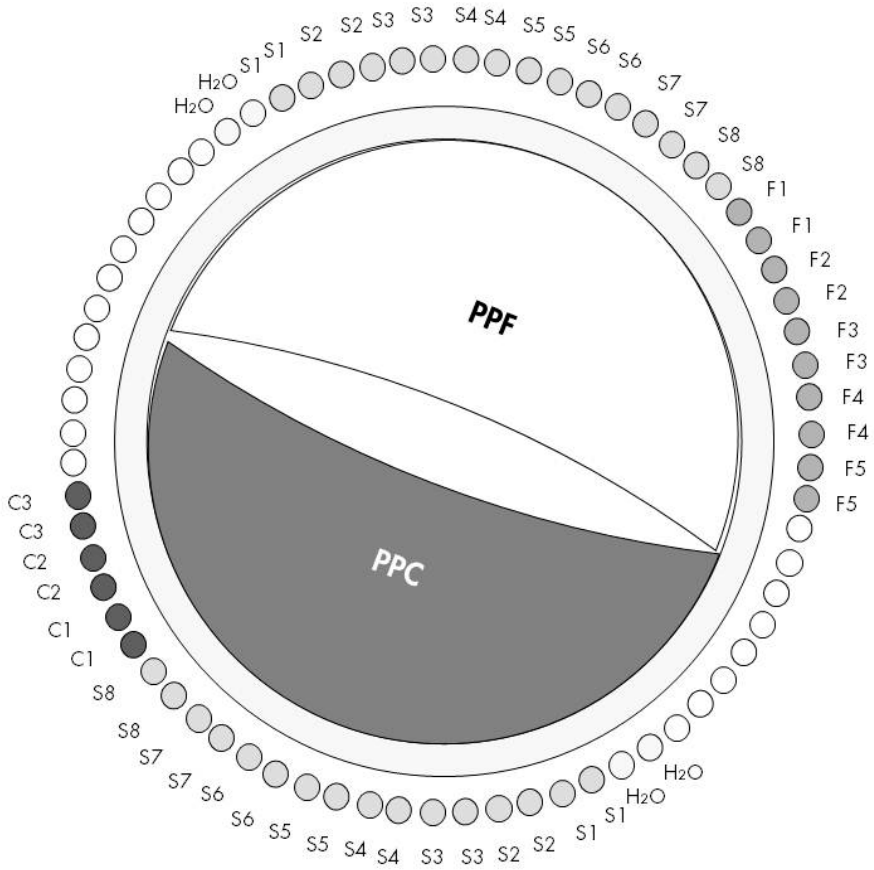

Figure 3. Suggested rotor setup for one experiment with an *ipsogen* FusionQ*uant* kit. **F1–5**: Fusion gene standards; **C1–3**: Control gene standards; **S**: cDNA sample; **H_2O**: water control.

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 4 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 primers and probe mix (either PPC or PPF). Extra volumes are included to compensate for pipetting error.

Table 4. Preparation of qPCR mix

Component	1 reaction (µl)	Control: 24+1 reactions (µl)	Fusion: 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: Reverse transcription", page 23) 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 5.**

Table 5. 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. **Start the thermal cycling program, as indicated in Table 5.**
9. **For Rotor-Gene Q instruments, select "Slope Correct" for the analysis. We recommend setting the threshold at 0.03.**

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 6.

Table 6. Number of reactions using 96-well-plate qPCR equipment

Samples	Reactions
With the control gene primers and probe mix (PPC)	
n cDNA samples	n x 2 reactions
Control gene standard	2 x 3 reactions (3 dilutions, each one tested in duplicate)
Water control	2 reactions
With the fusion gene primers and probe mix (PPF)	
n cDNA samples	n x 2 reactions
Fusion gene 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. Each *ipsogen* FusionQ*uant* kit provides enough reagents to perform an 8-sample experiment 3 times. The *ipsogen* BCR-ABL-Mbcr Kit for 52 reactions (cat. no. 670115) provides enough reagents to perform a 16-sample experiment 4 times.

The plate scheme in Figure 4 shows an example of an 8-sample experiment.

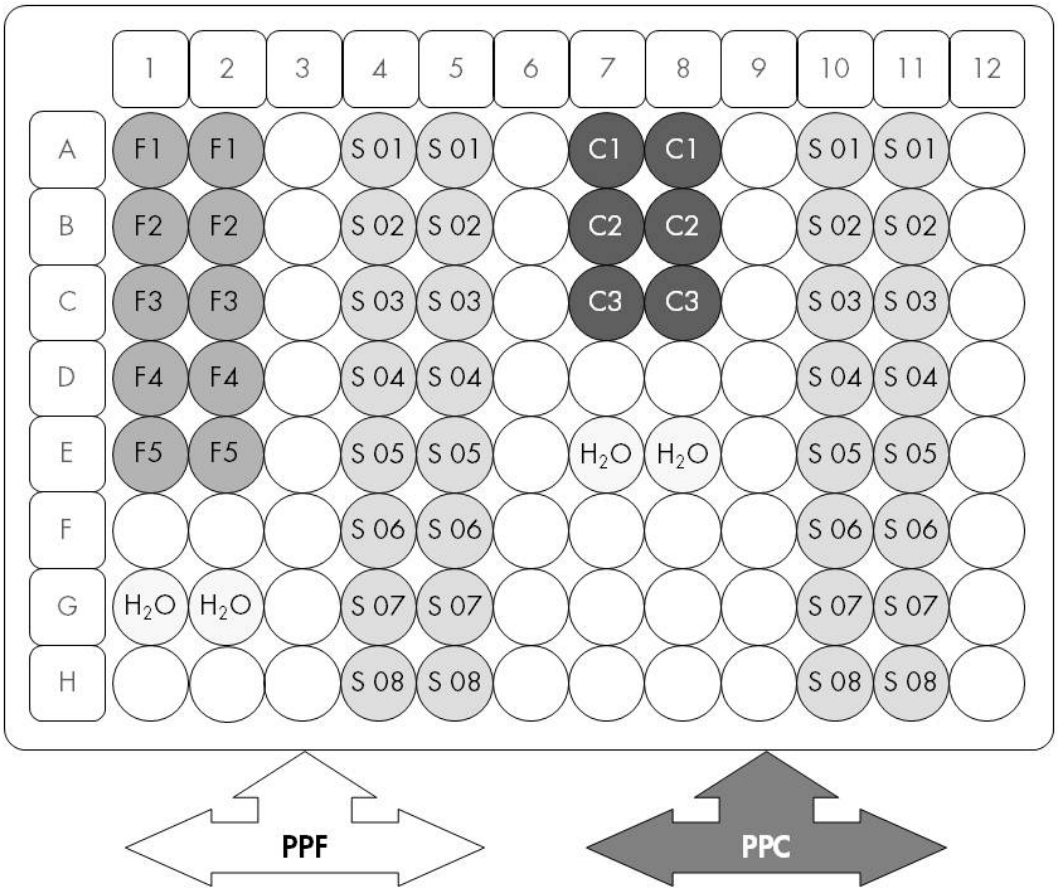

Figure 4. Suggested plate setup for one experiment. S: cDNA sample; **F1–5**: Fusion gene standards; **C1–3**: Control gene standards; **H_2O**: water control.

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 7 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 primers and probe mix (either PPC or PPF). Extra volumes are included to compensate for pipetting error.

Table 7. Preparation of qPCR mix

Component	1 reaction (µl)	Control: 24+1 reactions (µl)	Fusion: 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: Reverse transcription", page 23) 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 8 for ABI PRISM 7000, 7700, and 7900HT SDS, or Table 9 for the LightCycler 480 Instrument.**

Table 8. 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 9. 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 8.

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 9.

Protocol: qPCR on LightCycler 1.2, 1.5, and 2.0 Instruments

Using capillary instruments, we recommend measuring samples in duplicate and controls only once, as indicated in Table 10.

Table 10. Number of reactions for LightCycler 1.2, 1.5, and 2.0 Instruments

Samples	Reactions
With the control gene primers and probe mix (PPC)	
n cDNA samples	n x 2 reactions
Control gene standard	1 x 3 reactions (3 standard dilutions, each one tested once)
Water control	1 reaction
With the fusion gene primers and probe mix (PPF)	
n cDNA samples	n x 2 reactions
Fusion gene standard	1 x 5 reactions (5 standard dilutions, each one tested once)
Water control	1 reaction

Sample processing on LightCycler 1.2, 1.5, 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. Each *ipsogen* FusionQ*uant* kit provides enough reagents to perform a 5-sample experiment 6 times. The *ipsogen* BCR-ABL-Mbcr Kit for 52 reactions (cat. no. 670115) provides enough reagents to perform a 5-sample experiment 11 times.

The capillary scheme in Figure 5 shows an example of an experiment.

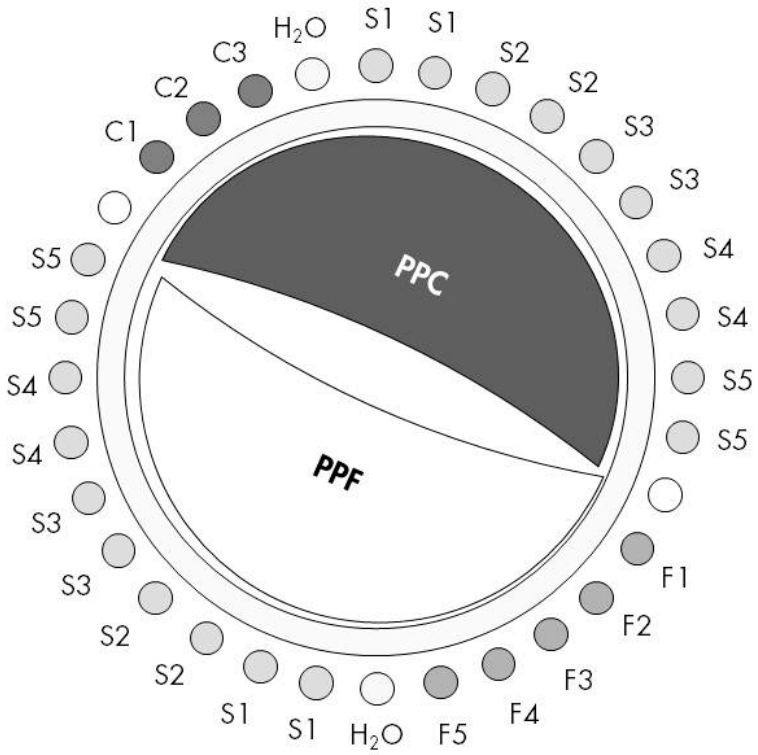

Figure 5. Suggested rotor setup for one experiment with an *ipsogen* FusionQ*uant* kit. **F1–5**: Fusion gene standards; **C1–3**: Control gene standards; **S**: unknown DNA sample to be analyzed; **H_2O**: water control.

qPCR on LightCycler 1.2, 1.5, 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 11 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 primers and probe mix (either PPC or PPF). Extra volumes are included to compensate for pipetting error.

Table 11. Preparation of qPCR mix

Component	1 reaction (µl)	Control: 14+1 reactions (µl)	Fusion: 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: Reverse transcription", page 23) 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, 1.5, or 2.0 Instruments with the thermal cycling program as indicated in Table 12.**

Table 12. 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 and 1.5, follow step 9a. For the LightCycler 2.0, follow step 9b.

9a. LightCycler 1.2 and 1.5: The F1/F2 and "2nd derivative analysis" mode is recommended. Start the thermal cycling program, as indicated in Table 12.

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 12.

Protocol: qPCR on the SmartCycler instrument

Using this instrument, we recommend measuring samples in duplicate and controls only once, as indicated in Table 13.

Table 13. Number of reactions for the SmartCycler instrument

Samples	Reactions
With the control gene primers and probe mix (PPC)	
n cDNA samples	n x 2 reactions
Control gene standard	1 x 3 reactions (3 standard dilutions, each one tested once)
Water control	1 reaction
With the fusion gene primers and probe mix (PPF)	
n cDNA samples	n x 2 reactions
Fusion gene 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. Each *ipsogen* FusionQ*uant* kit provides enough reagents to perform a 5-sample experiment 6 times. The *ipsogen* BCR-ABL-Mbcr Kit for 52 reactions (cat. no. 670115) provides enough reagents to perform a 5-sample experiment 11 times.

The two-block scheme in Figure 6 shows an example of one experiment.

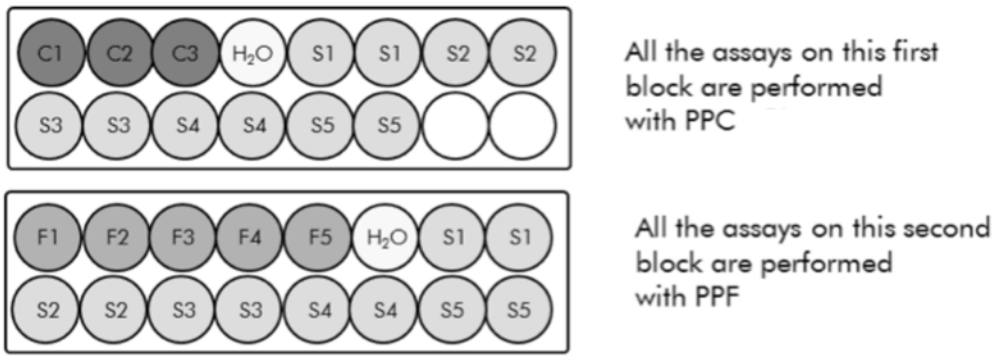

Figure 6. Suggested plate setup for one experiment. S: cDNA sample; **F1–5**: Fusion gene standards; **C1–3**: Control gene standards; **H_2O**: water control.

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 14 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 or PPF). Extra volumes are included to compensate for pipetting error.

Table 14. Preparation of qPCR mix

Component	1 reaction (µl)	Control: 14+1 reactions (µl)	Fusion: 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: Reverse transcription", page 23) 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 15.**

Table 15. 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 15.**

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 control gene, and 5 standard dilutions for the fusion gene, in order to ensure accurate standard curves. Figures 7 and 8 show an example of TaqMan amplification curves obtained with the *ipsogen* BCR-ABL Mbcr Kit (cat. no.670113).

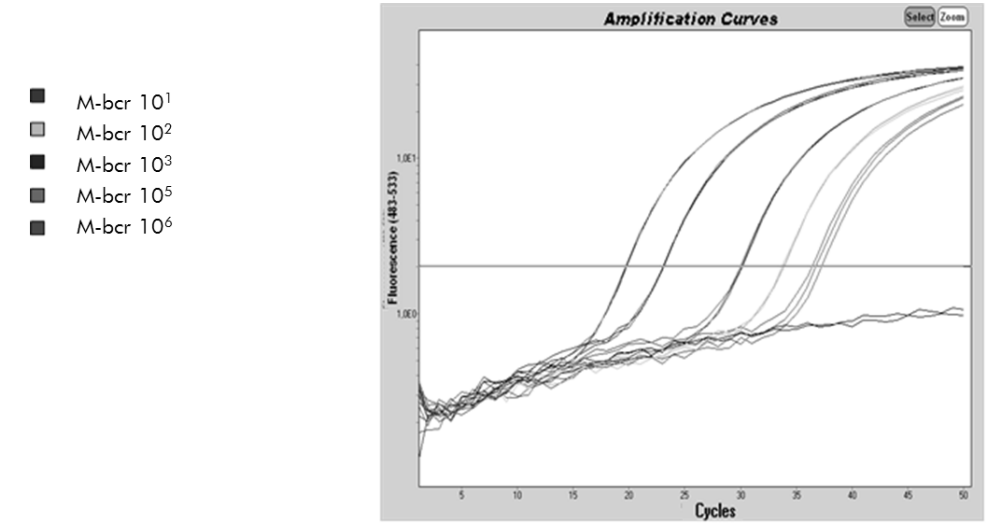

Figure 7. Detection of BCR-ABL Mbcr fusion gene standards (F1–F5). 10^1, 10^2, 10^3, 10^5, 10^6 copies/5 µl.

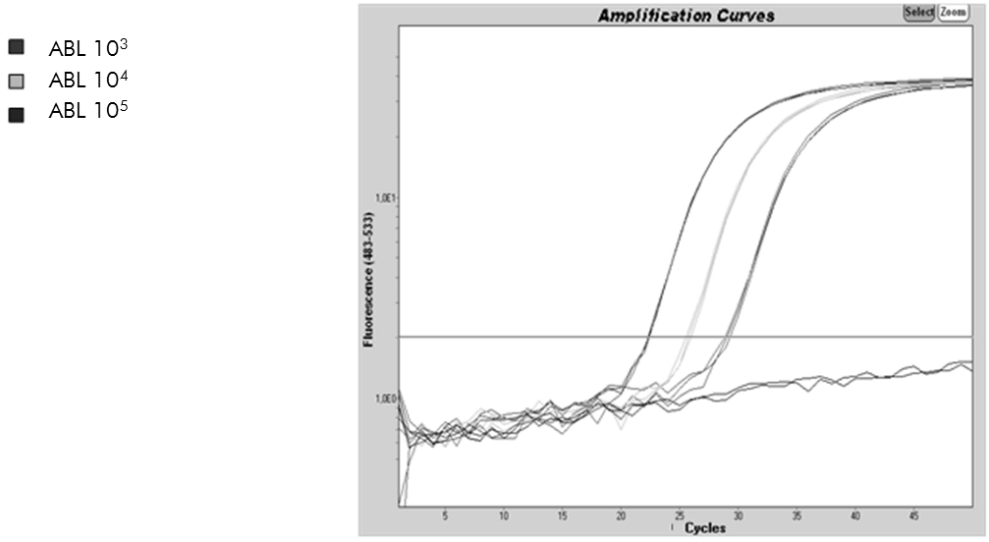

Figure 8. Detection of ABL control gene standards (C1, C2, C3). 10^3, 10^4, and 10^5 copies/5 µl.

Standard curve and quality criteria

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

For each gene (control gene and fusion gene), 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 9 shows an example of the theoretical curve calculated on 5 standard dilutions.

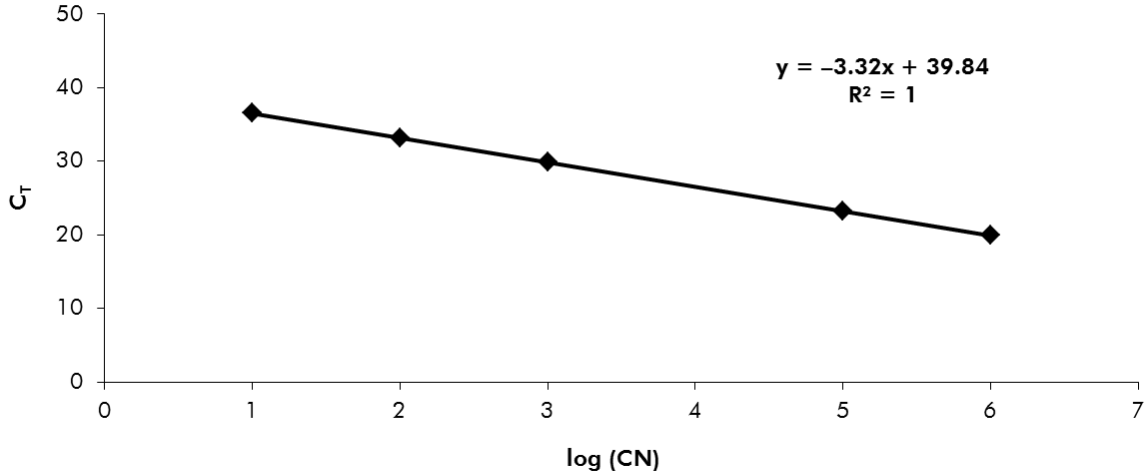

Figure 9. Theoretical curve calculated from the 5 standard dilutions. A linear regression curve (y = ax + b) is calculated for each gene (control gene and fusion gene), where a is the slope of the line and b is the y-intercept, which is the y-coordinate of the point where the line crosses the y axis. 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. However, a value for R^2 >0.98 is desirable for precise results.

Normalized copy number (NCN)

The control gene standard curve equation should be used to transform raw C_T values (obtained with PPC) for the unknown samples into control gene copy numbers (Control $gene_{CN}$).

The fusion gene standard curve equation should be used to transform raw C_T values (obtained with PPF) for the unknown samples, into fusion gene copy numbers (Fusion $gene_{CN}$).

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

$$NCN = \frac{\text{Fusion gene}_{CN}}{\text{Control gene}_{CN}}$$

Quality control on control gene values

Poor quality of the RNA or problems during the qPCR steps result in low Control gene_{CN}.

Reproducibility between replicates

The data obtained should be consistent between duplicates.

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 48).

	Comments and suggestions
Negative result for the control gene and fusion gene 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 (e.g., *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 (e.g., *ipsogen* BCR-ABL1 Mbcr Controls Kit, cat. no. 670191) in parallel.
Negative result for the control gene 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 (e.g., *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 (e.g., *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* FusionQ*uant* kits at –15 to –30°C and keep primers and probe mixes (PPC and PPF) protected from light. See "Reagent Storage and Handling", page 22. 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 (e.g., *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 (e.g., *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* FusionQ*uant* kits at –15 to –30°C and keep primers and probe mixes (PPC and PPF) protected from light. See "Reagent Storage and Handling", page 22. 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 standard. Certificates of analysis are available on request at www.qiagen.com/support/.

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.

Symbols

The following symbols may appear on the packaging and labeling:

 <N>	Contains reagents sufficient for <N> reactions
	Use by
	Catalog number
	Lot number
	Material number
	Global Trade Item Number
	Temperature limitation
	Manufacturer
	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	670013
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	670113
ipsogen BCR-ABL1 Mbcr Kit (52)	For 52 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	670115
ipsogen BCR-ABL1 mbcr (BCR) Kit (24)	For 24 reactions: BCR Control Gene Standards, BCR-ABL mbcr Fusion Gene Standards, Primer and Probe Mix BCR, Primer and Probe Mix BCR-ABL mbcr Fusion Gene	670213
ipsogen BCR-ABL1 Mbcr (BCR) Kit (24)	For 24 reactions: BCR Control Gene Standards, BCR-ABL Mbcr Fusion Gene Standards, Primer and Probe Mix BCR, Primer and Probe Mix BCR-ABL Mbcr Fusion Gene	670313
ipsogen BCR-ABL1 mbcr (GUS) Kit (24)	For 24 reactions: GUS Control Gene Standards, BCR-ABL mbcr Fusion Gene Standards, Primer and Probe Mix GUS, Primer and Probe Mix BCR-ABL mbcr Fusion Gene	670413
ipsogen BCR-ABL1 Mbcr (GUS) Kit (24)	For 24 reactions: GUS Control Gene Standards, BCR-ABL Mbcr Fusion Gene Standards, Primer and Probe Mix GUS, Primer and Probe Mix BCR-ABL Mbcr Fusion Gene	670513

Product	Contents	Cat. no.
ipsogen PML-RARA bcr1 Kit (24)	For 24 reactions: ABL Control Gene Standards, PML-RARA bcr1 Fusion Gene Standards, Primer and Probe Mix ABL, Primer and Probe Mix PML-RARA bcr1 Fusion Gene	672113
ipsogen PML-RARA bcr2 Kit (24)	For 24 reactions: ABL Control Gene Standards, PML-RARA bcr2 Fusion Gene Standards, Primer and Probe Mix ABL, Primer and Probe Mix PML-RARA bcr2 Fusion Gene	672213
ipsogen PML-RARA bcr3 Kit (24)	For 24 reactions: ABL Control Gene Standards, PML-RARA bcr3 Fusion Gene Standards, Primer and Probe Mix ABL, Primer and Probe Mix PML-RARA bcr3 Fusion Gene	672313
ipsogen RUNX1-RUNX1T1 Kit (24)	For 24 reactions: ABL Control Gene Standards, RUNX1-RUNX1T1 Fusion Gene Standards, Primer and Probe Mix ABL, Primer and Probe Mix RUNX1-RUNX1T1 Fusion Gene	675013
ipsogen ETV6-RUNX1 Kit (24)	For 24 reactions: ABL Control Gene Standards, ETV6-RUNX1 Fusion Gene Standards, Primer and Probe Mix ABL, Primer and Probe Mix ETV6-RUNX1 Fusion Gene	675113
ipsogen CBFB-MYH11A Kit (24)	For 24 reactions: ABL Control Gene Standards, CBFB-MYH11A Fusion Gene Standards, Primer and Probe Mix ABL, Primer and Probe Mix CBFB-MYH11A Fusion Gene	676013
Rotor-Gene Q — for outstanding performance in real-time PCR		
Rotor-Gene Q 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	9001580

Product	Contents	Cat. no.
Rotor-Gene Q 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	9001650
***ipsogen* Fusion Gene Controls kits — for qualitative testing of RNA extraction and reverse transcription of the fusion genes**		
ipsogen BCR-ABL1 mbcr Controls Kit	Cell lines with negative, high, and low positive expression of the BCR-ABL mbcr fusion gene	670091
ipsogen BCR-ABL1 Mbcr Controls Kit	Cell lines with negative, high, and low positive expression of the BCR-ABL Mbcr fusion gene	670191
ipsogen PML-RARA bcr1 Controls Kit	Cell lines with negative, high, and low positive expression of the PML-RARA bcr1 fusion gene	672091
***ipsogen* RT Kit — for reverse transcription**		
ipsogen RT Kit (33)	For 33 reactions: Reverse transcriptase, 5x RT buffer, dNTP mix, Random primer, RNase Inhibitor, DTT	679913

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Australia ■ techservice-au@qiagen.com

Austria ■ techservice-at@qiagen.com

Belgium ■ techservice-bnl@qiagen.com

Brazil ■ suportetecnico.brasil@qiagen.com

Canada ■ techservice-ca@qiagen.com

China ■ techservice-cn@qiagen.com

Denmark ■ techservice-nordic@qiagen.com

Finland ■ techservice-nordic@qiagen.com

France ■ techservice-fr@qiagen.com

Germany ■ techservice-de@qiagen.com

Hong Kong ■ techservice-hk@qiagen.com

India ■ techservice-india@qiagen.com

Ireland ■ techservice-uk@qiagen.com

Italy ■ techservice-it@qiagen.com

Japan ■ techservice-jp@qiagen.com

Korea (South) ■ techservice-kr@qiagen.com

Luxembourg ■ techservice-bnl@qiagen.com

Mexico ■ techservice-mx@qiagen.com

The Netherlands ■ techservice-bnl@qiagen.com

Norway ■ techservice-nordic@qiagen.com

Singapore ■ techservice-sg@qiagen.com

Sweden ■ techservice-nordic@qiagen.com

Switzerland ■ techservice-ch@qiagen.com

UK ■ techservice-uk@qiagen.com

USA ■ techservice-us@qiagen.com

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