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ipsogen® BCR-ABL1 Mbcr Controls Kit Handbook

For research use only. Not for use in diagnostic procedures

REF 670191

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANY

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QIAGEN Sample and Assay Technologies

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- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

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Contents

Intended Use

The *ipsogen* BCR-ABL1 Mbcr Controls Kit is 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.

This kit provides a qualitative control to monitor performances of BCR-ABL p210 transcript detection by quantitative PCR (qPCR). The BCR-ABL p210 Control Kit vials should be processed in parallel with the samples to be quantified. This product is intended to be used as an integral part of good laboratory practice.

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 of recombinant DNA experiments, or to other applicable guidelines.

Principle of the Procedure

The use of qPCR permits the 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 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. 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 reported fluorescence primarily by Förster-type energy transfer.

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 reported 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 1). This process occurs every cycle and does not interfere with the exponential accumulation of the 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, non-specific amplification is not detected. Thus, increase in fluorescence is directly proportional to the target amplification during PCR.

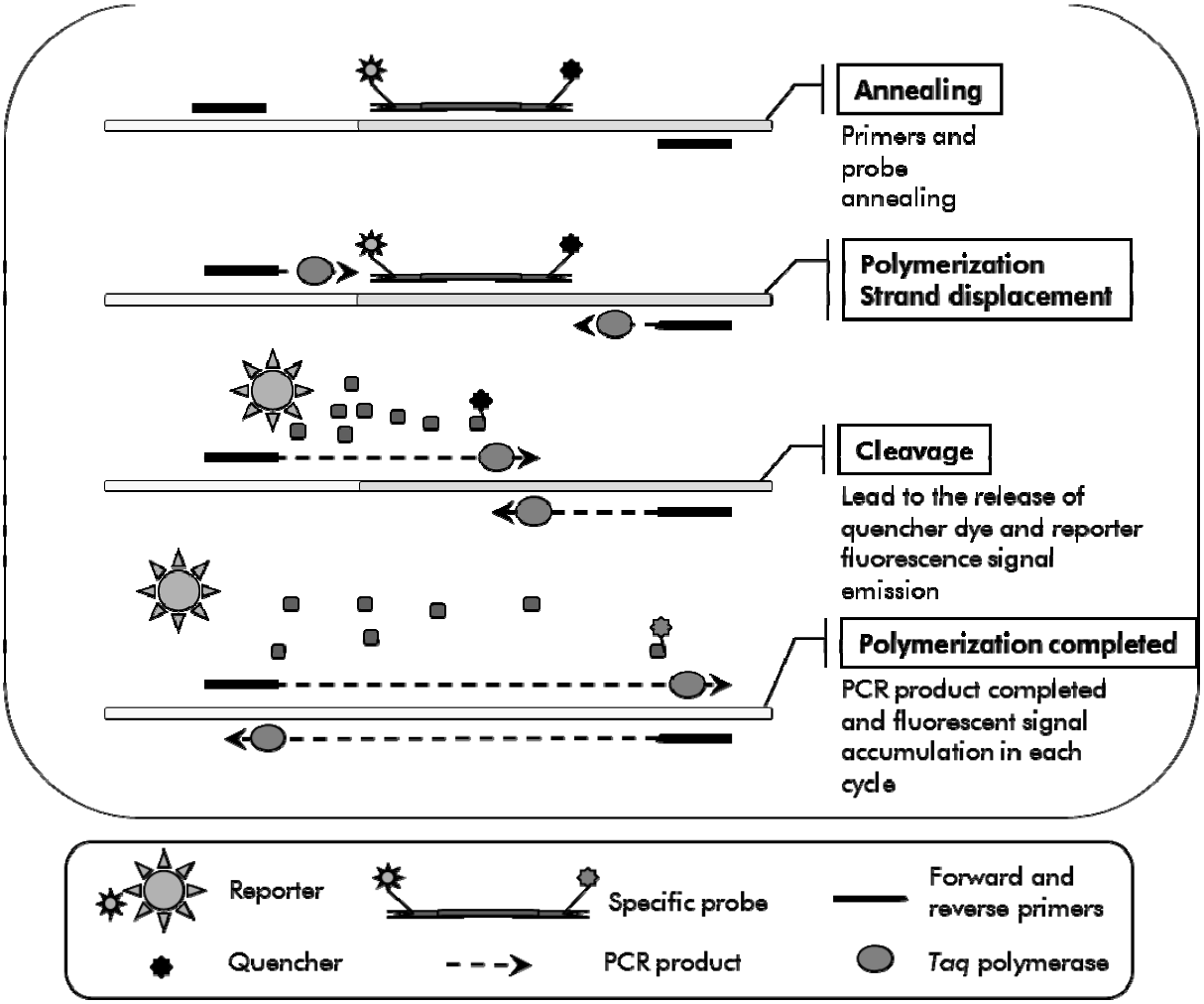

Figure 1. Reaction principle.

Materials Provided

Kit contents

***ipsogen* BCR-ABL1 Mbcr Controls Kit** **Catalog no.**		**670191**
High BCR-ABL p210 Control (5 x 10^6 cells in preservative solution)	High BCR-ABL1 p210 Control	1.0 ml
Low BCR-ABL p210 Control (5 x 10^6 cells in preservative solutions)	Low BCR-ABL1 p210 Control	1.0 ml
Negative BCR-ABL p210 & p190 Control (5 x 10^6 cells in preservative solutions)	Negative BCR-ABL1 p210 & p190 Control	1.0 ml
Dilution reagent (phosphate buffered saline)	PBS	3 x 0.6 ml
ipsogen *BCR-ABL1 Mbcr Controls important note*		1

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

- RNeasy® Mini Kit (QIAGEN, cat. no. 74104) or TRIzol® (Life Technologies, cat no. 15596-026)
- Chloroform
- Isopropyl alcohol
- 70% ethanol (in DEPC-treated water)
- Kit reagents and supplies for RNA Nano LabChip® (Agilent, cat. no. 5065-4476)
- RNA 6000 ladder
- RNA Nano Labchip (Agilent, cat. no. 5065-4475)
- Molecular weight markers
- Agarose
- Nuclease-free PCR grade water

Consumables

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

Equipment

- Microliter pipet* dedicated for RNA (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 13,000 to 14,000 rpm)
- Biophotometer
- Agilent Bioanalyzer® and associated material
- Electrophoresis chamber

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

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.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

General precautions

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

This kit is intended for research use. Reagents and instructions supplied in this kit 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. All reagents are formulated specifically for use with this kit. For optimal performance of the procedure, no substitutions should be made. Differences in sample processing and technical procedures in the user's laboratory may invalidate the assay results.

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.

- Minimize microbial contamination of reagents to avoid non-specific reactions
- Incubation times, temperatures, or methods other than those specified may give erroneous results

Ribonucleases (RNAses) are very stable and active enzymes. They are difficult to inactivate and even minute amounts are sufficient to destroy RNA. Great care should be taken to avoid inadvertently introducing RNAses in a sample during or after the isolation procedure. The following precautions must be taken during treatment to create and maintain an RNAse-free environment.

- Proper microbiological, aseptic-like technique should always be used when working with RNA
- Always wear latex or vinyl gloves to prevent RNase contamination from the surface of the skin
- Changes gloves frequently and more particularly after touching skin, doorknobs and common surfaces
- Use a dedicated set of pipets devoted solely for RNA work
- It is recommended to use disposable tips and tubes that are tested and guaranteed to be RNase-free. If non-disposable glassware, metalware or plasticware are used, they should be treated before use to ensure that this material is RNase-free. Plasticware should be rinsed with 0.1M NaOH, 1mM EDTA follow by RNase-free water or alternatively treated with a ready to use solution such as RNaseZap® (Ambion). Glassware could also be cleaned with detergent, thoroughly rinsed and oven baked at 240°C for 4 hours. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware could be treated with DEPC (diethyl pyrocarbonate). As DEPC is a suspect carcinogen, it should be handled with great care. Wear gloves and use a fume hood when using this chemical.
- Use RNAse-free chemicals and reagents
- Designate a "low-traffic" area as an "RNAse-free zone"

Reagent Storage and Handling

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

- 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*

Total RNA extraction

Procedure for recovery of the cell pellet

1. **Thaw each vial or room temperature. The controls may be frozen or in a liquid form, with or without crystals and without quality degradation.**
2. **Add 0.5 ml dilution reagent and homogenize by pipetting several times to dissolve any crystals that may be present in the tube.**
3. **Centrifuge the samples at 4000 *g* for 5 minutes.**
4. **Remove the supernatant by aspiration without disrupting the pellet.**

Procedure for preparation of the RNA

1. **Once cells have been recovered, immediately continue with your usual laboratory protocol for RNA extraction.**
2. **Measure the RNA concentration in each sample and quantify RNA quality using one of the methods described in the section on RNA quantification.**

Procedure for cDNA synthesis

1. **Prepare cDNA using 1.0 µg of total RNA**
2. **Use your own laboratory protocol to synthesize cDNA or refer to the recommended procedure described in the ipsogen BCR-ABL1 Mbcr Kit (24) (QIAGEN, cat. no. 670123 or 670113) handbook.**

Note: To use this kit as a consistent quality control for the RNA isolation step, one vial should be systematically processed in parallel of each batch of samples, from RNA purification to qPCR experiment.

* 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 for the product supplier.

RNA quantification

Fluorescent dye

The fluorescent dye, RiboGreen® (RiboGreen, Molecular Probe Inc.,) is a nucleic acid stain that can be used to quantify RNA in solution. The fluorescence of a sample containing RiboGreen is measured in a fluorometer and compared against a standard curve. This method is more sensitive than spectrophotometry and less sensitive to both protein and free nucleotide interference. However, it requires a fluorometer and DNA contamination can cause inaccurate quantification.

Spectrophotometry

Absorbance at 260 nm in a spectrophotometer (A_{260}) using a quartz cuvette is the traditional method of quantifying RNA. It is widely used, simple, and uses equipment that is commonly available in a laboratory. This method is, however, considerably less sensitive than other methods and DNA, protein and free nucleotides will interfere with the reading. The absorbance of a diluted RNA sample is measured at 260 and 280 nm. The nucleic acid concentration is calculated using the Beer-Lambert law, which predicts a linear change in absorbance with concentration. Using this equation, an A_{260} reading of 1.0 is equivalent to a concentration of ~40 µg/ml single-stranded RNA.

Agilent Bioanalyzer

The Agilent Bioanalyzer 2100 (Agilent Technologies) uses microfluidic/capillary electrophoresis to analyze nucleic acids with a RNA Nano LabChip. Quantification is accomplished by comparing fluorescence of the sample against a standard RNA 6000 ladder. It requires a small amount of RNA, can be used to quantify RNA, and assess RNA integrity. This method is not affected by common contaminants, but this instrument is not available in many laboratories.

RNA qualification

Taking into account the reduced size of amplicon, qPCR is tolerant of partially degraded RNA, but assessing the integrity of an RNA sample is an important aspect of gene expression analysis. When comparing samples it is important that the RNA samples show similar integrity. Varying degrees of degradation could introduce inconsistencies in the downstream results.

Denaturing gel electrophoresis

A 2:1 ratio of 28S (5.0 kb) to 18S (1.9 kb) on a denaturing gel indicates intact RNA, but this requires at least 100 ng of total RNA for visualization. Smeared

28S and 18S rRNA bands indicate degradation and the 28S to 18S ratio is will be under 2.

UV spectrometry

The ratio of the absorbance reading at 260 nm and 280 nm (A_{260}/A_{280}) is indicative of the purity of the sample. A ratio between 1.8 and 2.1 is indicative of highly purified RNA. This method does have several drawbacks, but they can be minimized by following these tips:

- As this method does not discriminate between RNA and DNA, it is advisable to first treat the RNA samples with RNase-free DNase to remove any contaminating DNA.
- Contaminants such as residual proteins and phenol can interfere with absorbance readings and care must be taken during RNA purification to remove these impurities.
- The A_{260}/A_{280} ratio is dependent on both pH and ionic strength. As pH increases, the A_{280} reading decreases while the A_{260} reading is unaffected. This results in an increasing A_{260}/A_{280} ratio. Water, which often has an acidic pH, can lower the A_{260}/A_{280} ratio and it is therefore recommended that a buffered solution with a slightly alkaline pH, such as TE at a pH of 8.0 is used as a diluent (and as a blank) to ensure accurate and reproducible readings. Pure RNA should have an A_{260}/A_{280} ratio of ~2. RNA with a significantly lower ratio should be further purified by phenol-chloroform extraction, precipitation, or washing to remove residual salts. Contaminating chemicals, proteins, and other molecules in RNA samples can inhibit or interfere with downstream applications.
- The RNA dilution must be within the linear range of the spectrophotometer being used. Usually absorbance values should fall between 0.1 and 1.0. If the solution has an absorbance outside of this range, the concentration cannot be assessed accurately. Generally, the greatest error occurs at low concentrations.

Agilent Bioanalyzer

This requires the sample to contain at least 25 ng RNA. Degradation is indicated by less pronounced peaks for 28S and 18S rRNA and the ratio is significantly under 2.

Figures 2 to 4 show traces typically seen on an Agilent Bioanalyzer for high-quality RNA, partially degraded total RNA, and degraded total RNA.

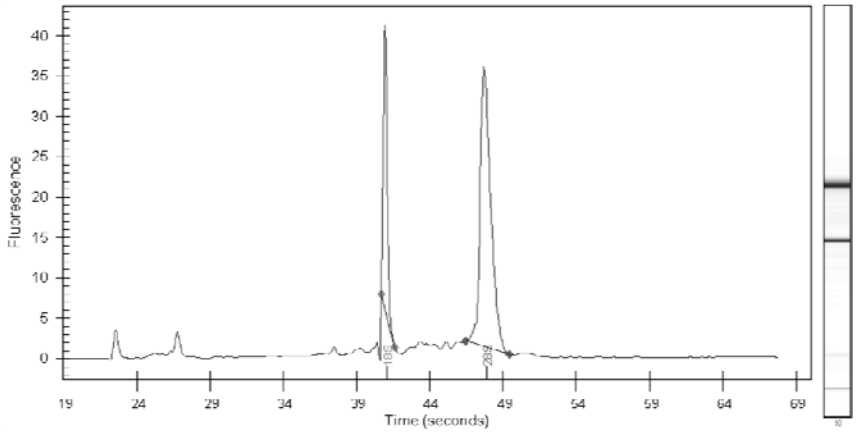

Figure 2. High-quality total RNA. rRNA ratio (28S/18S) is 2.16.

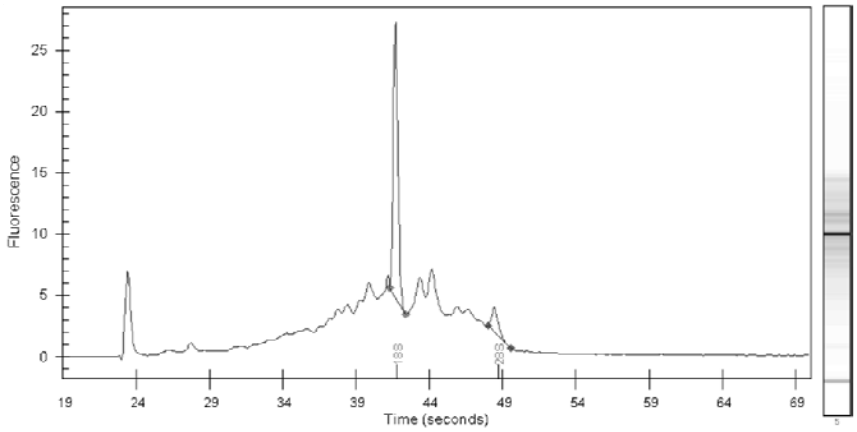

Figure 3. Partially degraded total RNA. rRNA ratio (28S/18S) is 0.11.

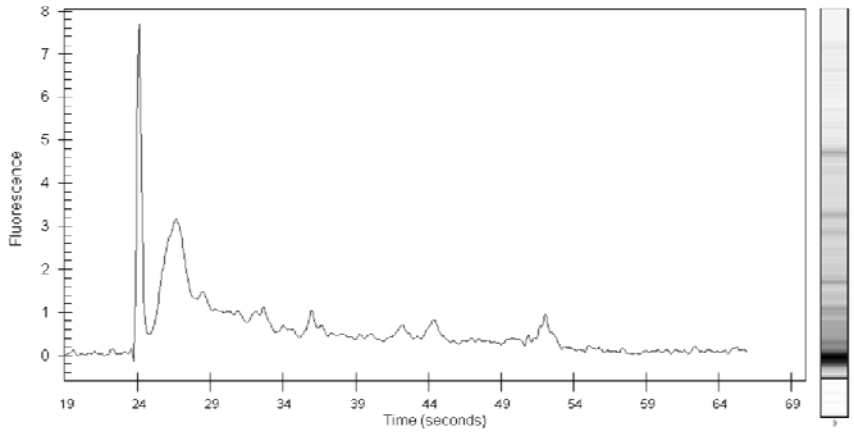

Figure 4. Degraded total RNA. rRNA ratio (28S/18S) is 0.00.

Identification of genomic DNA contamination

The presence of genomic DNA is a contaminant in the sample can lead to inaccurate RNA quantification. This can be assessed by denaturing gel electrophoresis or qPCR.

Denaturing gel electrophoresis

When the samples are ran on an agarose gel under denaturing electrophoresis conditions, any DNA contamination appears as high molecular weight band or smear.

qPCR

This is an easy way to check for DNA contamination. When PCR is performed using an RNA sample that has not been reverse transcribed, the presence of an amplified product indicates the presence of DNA contamination.

Expected Results

The values included in Tables 1 and 2 are only indicative of results and should not be considered as reference values.

Table 1. RNA extraction yield

Extraction method	Mean (95% CI) for high control	Mean (95% CI) for low control	Mean (95% CI) for negative control
TRIzol reagent	31 µg (8.1–54.8) n=102	15 µg (9.6–20.3) n=4	ND
RNeasy Mini Kit	24 µg (7.9–34.0) n=9	22 µg (14.5–30.8) n=21	25 µg (13.4–36.7) n=4

CI: confidence interval; n: number of samples; ND: Not determined.

Table 2. RNA quantification of high control

	CN_{Mbcr} (n=96)	CN_{ABL} (n=96)	NCN (n=70)
Mean	352,609	282,000	133
Range (minimum-maximum)	68,987-786,074	80,942-647,698	45-298

CN: copy number; n: number of samples; NA: not available; NCN: normalized copy number (CN_{Mbcr}/CN_{ABL} x 100).

Table 3. RNA quantification of low control

	CN_{Mbcr} (n=74)	CN_{ABL} (n=74)	NCN (n=30)
Mean	96	76,301	0.13
Range (minimum-maximum)	25-189	24,137-170,941	0.08-0.22

CN: copy number; n: number of samples; NA: not available; NCN: normalized copy number (CN_{Mbcr}/CN_{ABL} x 100).

Table 4. RNA quantification of negative control

	CN_{ABL} (n=39)
Mean	71,702
Range (minimum-maximum)	42,370-101,785

CN: copy number; n: number of samples; NA: not available; NCN: normalized copy number (CN_{Mbcr}/CN_{ABL} x 100).

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 18).

	Comments and suggestions
Low yield RNA	
a) Incomplete homogenization or lysis of the sample	Repeat but reduce the amount of starting material and/or increase the volume of lysis buffer and homogenization time.
b) RNA still bound on the membrane (RNeasy Mini Kit)	Repeat elution after incubating the column for 10 minutes with RNase-free water.
c) Incomplete dissolution of crystal before lysis	After dilution with PBS, ensure complete homogenization by pipetting several times to dissolve any crystals that may be present in the solution.
d) Cells not pelleted completely or efficiently	Check the centrifugal force applied to pellet the cells as this should be at least 3000 *g*.
e) Final RNA pellet not completely redissolved	Redissolve the RNA pellet in 10mM TrisCl, pH7.5.
High C_T value	
Poor reverse transcription or poor RNA quality/purity	Check RNA quality and purity using gel electrophoresis, the OD_{260}/OD_{280} ratio or an Agilent Bioanalyzer. Repeat the whole experiment from the reverse transcription step. Use new reverse transcription reagents.
RNA degraded	
a) Incorrect storage conditions	Store the *ipsogen* BCR-ABL Mbcr Controls at –15 to –30°C until used. See "Reagent Storage and Handling", page 9.
b) RNase contamination	Take care not to introduce any RNase during the procedure or during the subsequent handling. Use only RNase-free aqueous solutions or tubes.
DNA contamination	
Homogenization in too small a volume	Perform a DNase digestion using an RNase-free DNase (QIAGEN RNase-free DNase set, cat. no. 79254).

Quality Control

This kit is manufactured according to ISO 13485:2003 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.

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Symbols

	Use by
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	Temperature limitation
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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-ABL Mbcr Controls	High, low and negative controls for the qualitative validation of RNA extraction and reverse transcription steps in qPCR detection of BCR-ABL p210 (Mbcr) transcripts	670191
ipsogen BCR-ABL1 Mbcr Kit (24)	For 24 reactions: ABL Control Gene Standards, BCR-ABL Mbcr Fusion Gene Standards, Primers and Probe Mix ABL, Primers and Probe Mix BCR-ABL Mbcr Fusion Gene	670123
ipsogen BCR-ABL1 Mbcr Kit (24)	For 24 reactions: ABL Control Gene Standards, BCR-ABL Mbcr Fusion Gene Standards, Primers and Probe Mix ABL, Primers and Probe Mix BCR-ABL Mbcr Fusion Gene	670113

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Notes

Notes

Notes

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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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