January 2013

ipsogen® MN1 *Profile*Quant® Handbook

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For research use only. Not for use in diagnostic procedures

For use with Rotor-Gene® Q 5plex HRM®, ABI PRISM® 7700 and 7900HT SDS, Applied Biosystems® 7500 Real-Time PCR System, LightCycler® 480 and 1.2 instruments

REF 676813

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Intended Use

The *ipsogen* MN1 Profile*Quant* 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.

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, nonspecific amplification is not detected. Thus, increase in fluorescence is directly proportional to the target amplification during PCR.

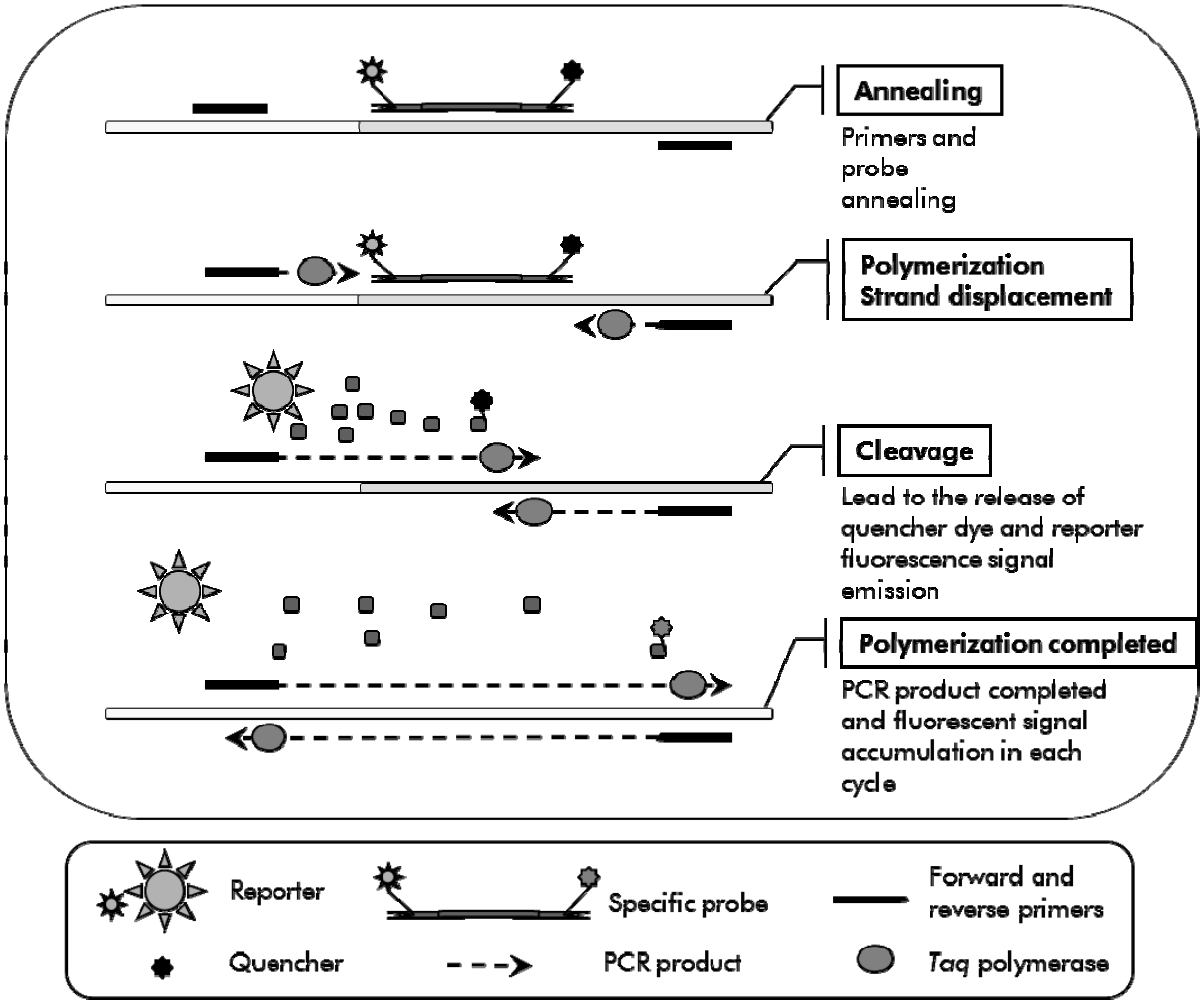

Figure 1. Reaction principle.

Materials Provided

Kit contents

***ipsogen* MN1 Profile *Quant* Kit**		**(24)**
Catalog no.		**676813**
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
MN1 Profile Standard Dilution (10^1 copies/5 µl)	P1-MN1	50 µl
MN1 Profile Standard Dilution (10^2 copies/5 µl)	P2-MN1	50 µl
MN1 Profile Standard Dilution (10^3 copies/5 µl)	P3-MN1	50 µl
MN1 Profile Standard Dilution (10^5 copies/5 µl)	P4-MN1	50 µl
MN1 Profile Standard Dilution (10^6 copies/5 µl)	P5-MN1	50 µl
Primers and Probe Mix ABL*	PPC-ABL 25x	90 µl
Primers and Probe Mix MN1†	PPP-MN1 25x	110 µl
ipsogen *MN1 Profile*Quant *important note*		1

* 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 *MN1* gene plus a specific FAM–TAMRA probe.

Note: Vortex and 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® III Reverse Transcriptase, includes 5x first-strand buffer, 100 mM DTT (Life Technologies, cat. no. 18080-044)
- RNase inhibitor: The recommended reagent is RNaseOUT™ (Life Technologies, cat. no. 10777-019)
- Set of dNTPs, PCR grade
- Random nonamer
- 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 nuclease-free PCR tubes
- 0.1 ml strip tubes and caps if using the Rotor-Gene Q 5plex HRM instrument
- 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 13,000 to 14,000 rpm)
- Real-time PCR instrument:* Rotor-Gene Q 5plex HRM; ABI PRISM 7700 or 7900HT SDS; or Applied Biosystems 7500 Real-Time PCR System; LightCycler 480 and 1.2 instruments; and associated specific material

* 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. PPC and PPP reagents may be altered if exposed to light. All reagents are formulated specifically for use with this kit. For optimal performance of the kit, 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 P1–5) 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 PPP 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

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 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. **Mix well (do not vortex) and spin briefly (10,000 rpm for 10 seconds) to collect the liquid at the bottom of the tube.**
3. **Adjust RNA samples to 0.1 µg/µl.**
4. **Incubate 1 µg of RNA samples to be tested (10 µl) for 5 minutes at 65°C and immediately cool on ice for 5 minutes.**
5. **Centrifuge briefly (approximately 10 seconds, 10,000 rpm, to collect the liquid in the bottom of the tube). Then keep on ice.**
6. **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. For more information, consult the appropriate safety data sheets (SDSs) available for the product supplier.

Table 1. Preparation of RT mix

Component	Volume per sample (µl)	Final concentration
Reverse Transcription Buffer, 5x	5.0	1x
dNTPs (10 mM each, to be prepared previously and stored at –20°C in aliquots)	2.0	0.8 mM
DTT	1.25	–
RNase inhibitor (40 U/µl)	0.5	0.8 U/µl
Random nonamer (100 µM)	5.25	21 µM
Reverse Transcriptase (200 U/µl)	1.0	8 U/µl
RNA sample (to be added in step 5)	10.0	40 ng/µl
Final volume	25.0	–

7. Pipet 15 µl of RT mix into each PCR tube and add 10 µl (1 µg) RNA (from step 3) as shown in Table 2.

Table 2. Preparation of reverse transcription reaction

Component	Volume (µl)
RT mix	15
Heated sample RNA (1 µg)	10
Final volume	25

8. Mix with care and centrifuge briefly (approximately 10 seconds, 10,000 rpm, to collect the liquid in the bottom of the tube).
9. Incubate at 25°C for 10 minutes.
10. Incubate at 50°C for 60 minutes and then immediately at 85°C for 5 minutes.
11. Cool on ice (to stop the reaction) for 5 minutes.
12. Briefly centrifuge (approximately 10 seconds, 10,000 rpm, to collect the liquid in the bottom of the tube). Then keep on ice.
13. Carry out PCR according to the following protocols, according to your qPCR instrument.

Protocol: qPCR on Rotor-Gene Q 5plex HRM Instruments

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 with 0.1 ml strip tubes and caps

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 MN1 primers and probe mix (PPP-MN1)	
n cDNA samples	n x 2 reactions
MN1 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 with 0.1 ml strip tubes and caps

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* MN1 Profile*Quant* Kit provides enough reagents to perform an 8-sample experiment 3 times using the 72-tube rotor.

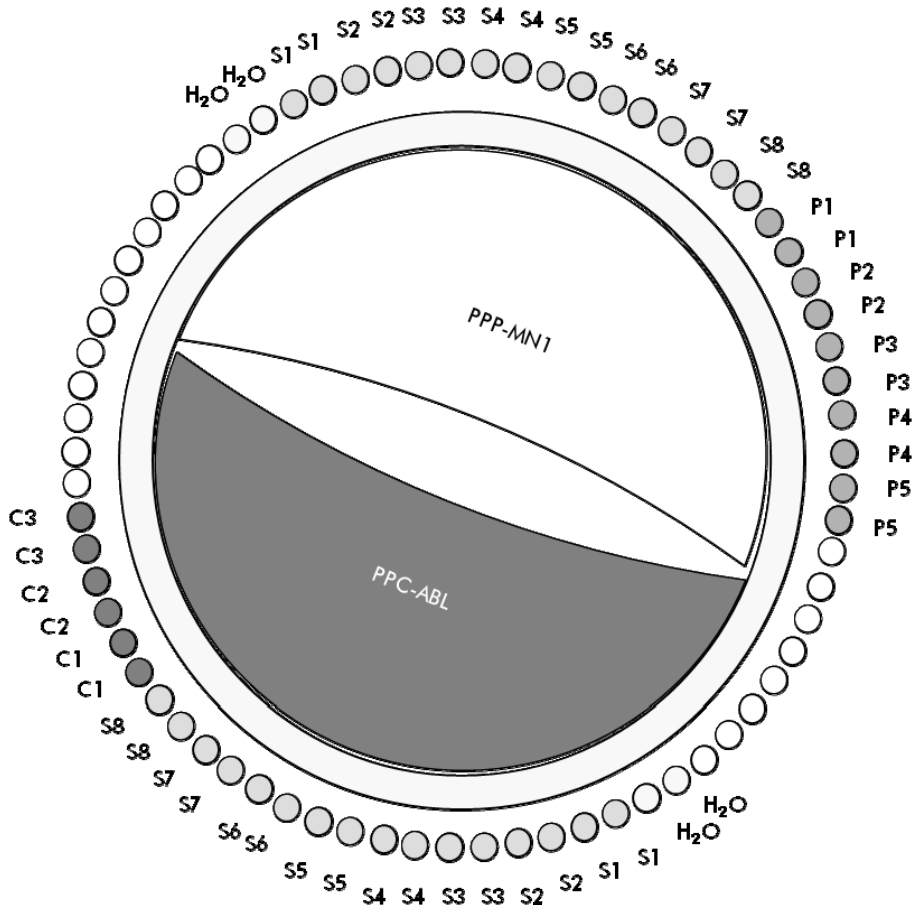

Figure 2. Suggested rotor setup for each experiment with the *ipsogen* MN1 Profile*Quant* Kit. P1–5: MN1 standards; **C1–3**: ABL standards; **H_2O**: water control; **S**: cDNA sample.

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 with 0.1 ml strip tubes and caps

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 primer

and probe mix (either PPC-ABL or PPP-MN1). Extra volumes are included to compensate for pipetting error.

Table 4. Preparation of qPCR mix

Component	1 reaction (µl)	ABL: 24 + 1 reactions (µl)	MN1: 28 + 1 reactions (µl)	Final concentration
TaqMan Universal PCR Master Mix, 2x	12.5	312.5	362.5	1x
Primers and probe mix, 25x	1.0	25.0	29.0	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, 200 ng RNA equivalent) obtained in the reverse transcription (see "Protocol: Recommended reverse transcription", page 10) 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 1	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 5.**

Protocol: qPCR on ABI PRISM 7700 and 7900HT SDS Instruments, the Applied Biosystems 7500 Real-Time PCR System, and the 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 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 MN1 primers and probe mix (PPP-MN1)	
n cDNA samples	n x 2 reactions
MN1 standard	2 x 5 reactions (5 dilutions, each one tested in duplicate)
Water control	2 reactions

Sample processing on ABI PRISM 7700 and 7900 SDS instruments, the Applied Biosystems 7500 Real-Time PCR System, and the LightCycler 480

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 3 shows an example of such an experiment.

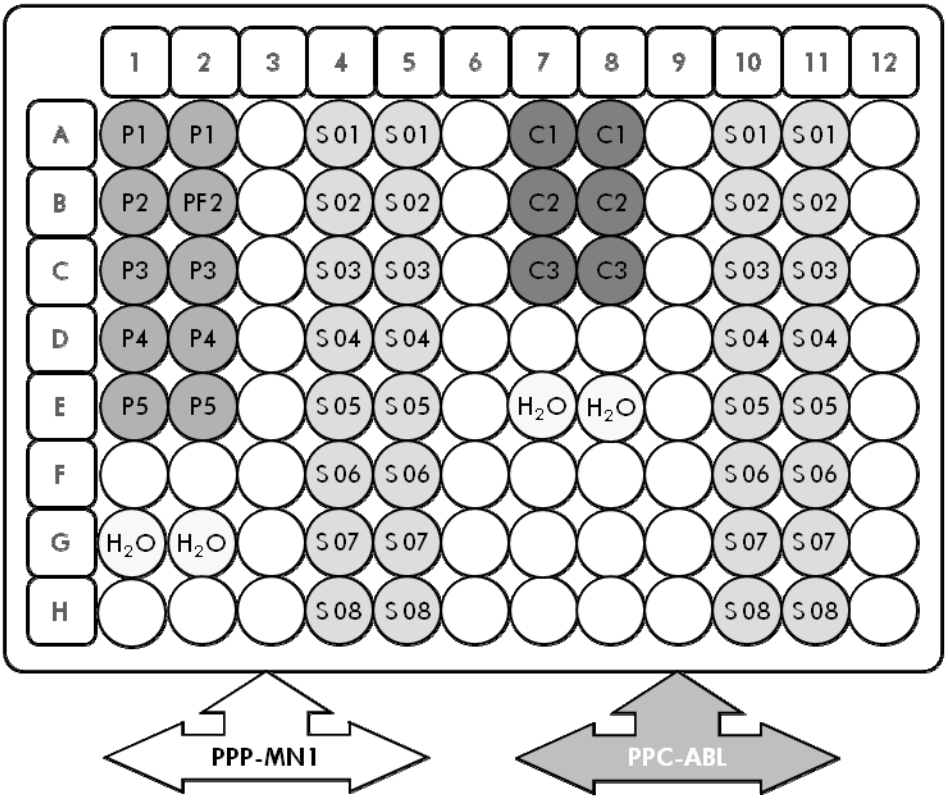

Figure 3. Suggested plate setup for one experiment. P1–5: MN1 standards; **C1–3**: ABL standards; **H_2O**: water control; **S**: cDNA sample.

qPCR on ABI PRISM 7700 and 7900 SDS instruments, the Applied Biosystems 7500 Real-Time PCR System, and the LightCycler 480

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-ABL or PPP-MN1). Extra volumes are included to compensate for pipetting error.

Table 7. Preparation of qPCR mix

Component	1 reaction (µl)	PPC-ABL: 24 + 1 reactions (µl)	PPP-MN1: 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	25.0	29.0	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, 200 ng RNA equivalent) obtained in the reverse transcription (see "Protocol: Recommended reverse transcription", page 10) 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.**
8. **Program the thermal cycler with the thermal cycling program and set the instrument for the acquisition of dual labeled FAM fluorescent probe as indicated in Table 8 for the ABI PRISM 7700 and 7900HT SDS instruments and Applied Biosystems 7500 Real-Time PCR System, or Table 9 for the LightCycler 480 instrument.**

Table 8. Temperature profile for ABI PRISM 7700 and 7900HT SDS instruments and the Applied Biosystems 7500 Real-Time PCR System

Mode of analysis	Standard Curve — Absolute Quantitation
Hold 1	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 the LightCycler 480 instrument

Mode of analysis	Absolute Quantification (Abs Quant)
Hold 1	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 7700 and 7900HT SDS instruments and the Applied Biosystems 7500 Real-Time PCR System, follow step 8a. For the LightCycler 480 instrument, follow step 8b.

8a. ABI PRISM 7700 and 7900HT SDS instruments and the Applied Biosystems 7500 Real-Time PCR System: We recommend a threshold set at 0.1 and a baseline set between cycles 3 and 15. Start the thermal cycling program, as indicated in Table 8.

8b. LightCycler 480: 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 Instrument

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 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 MN1 primers and probe mix (PPP-MN1)	
n cDNA samples	n x 2 reactions
MN1 standard	1 x 5 reactions (5 standard dilutions, each one tested once)
Water control	1 reaction

Sample processing on LightCycler 1.2 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 capillary scheme in Figure 4 shows an example of an experiment.

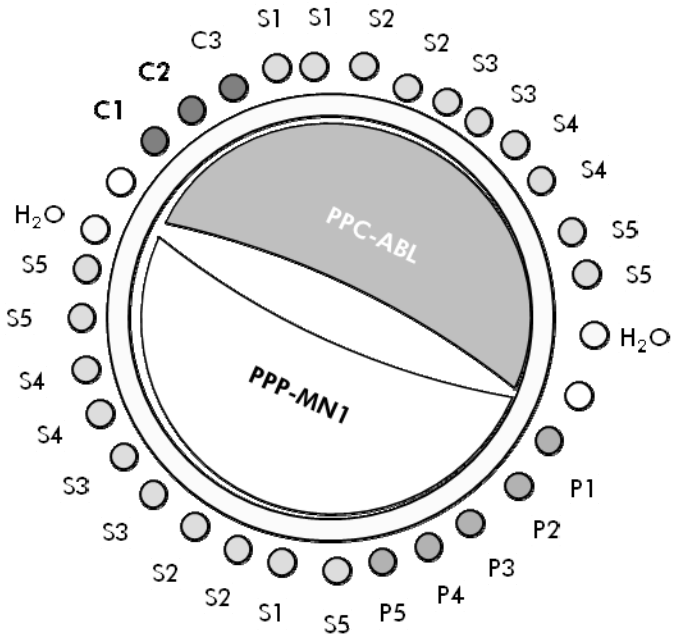

Figure 4. Suggested rotor setup for each experiment with the *ipsogen* MN1 Profile*Quant* Kit. P1–5: MN1 standards; **C1–3**: ABL standards; **H_2O**: water control; **S**: unknown DNA sample to be analyzed.

qPCR on LightCycler 1.2 instrument

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 10 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-ABL or PPP-MN1). Extra volumes are included to compensate for pipetting error.

Table 11. Preparation of qPCR mix

Component	1 reaction (µl)	ABL: 14+1 reactions (µl)	MN1: 16+1 reactions (µl)	Final concentration
Freshly prepared LightCycler TaqMan Master Mix, 5x	4.0	60.0	68.0	1x
Primers and probe mix, 25x	0.8	12.0	13.6	1x
Nuclease-free PCR grade water	10.2	153.0	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	–

4. **Dispense 15 µl of the qPCR pre-mix per capillary.**
5. **Add 5 µl of the RT product (cDNA, 200 ng RNA equivalent) obtained in the reverse transcription (see "Protocol: Recommended reverse transcription", page 10) in the corresponding tube (total volume 20 µl).**
6. **Mix gently, by pipetting up and down.**
7. **Place the capillaries in the adapters provided with the instrument, and briefly centrifuge (700 x *g*, approximately 10 seconds).**
8. **Load the capillaries into the thermal cycler according to the manufacturer's recommendations.**
9. **Program the LightCycler 1.2 instrument 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

10. For the LightCycler 1.2, we recommend the F1/F2 and "2nd derivative analysis" mode is selected. Start the thermal cycling program, as indicated in Table 12.
■

Results

Data analysis principle

Data for the threshold cycle (C_T) and crossing point (C_P) values can be exported from the qPCR instrument and pasted into an Excel® file for analysis. These values can then be used to calculate the mean value for C_T and C_P and the standard mean C_T values can be plotted to obtain a standard curve.

Standard curve and quality criteria

Figures 6 and 7 show examples of TaqMan amplification curves obtained with the *ipsogen* MN1 Profile*Quant* Kit and Figure 8 shows an example of the theoretical standard curve.

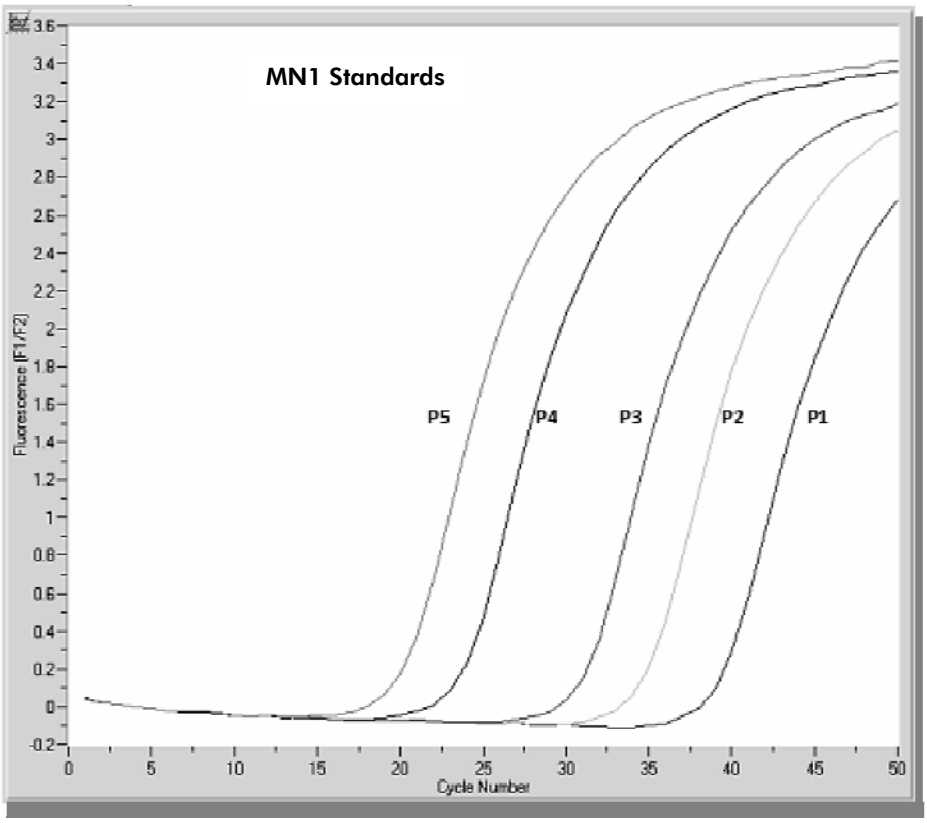

Figure 6. Amplification plot of MN1 standards (P1–P5). 10^1, 10^2, 10^3, 10^5, and 10^6 copies/5 µl.

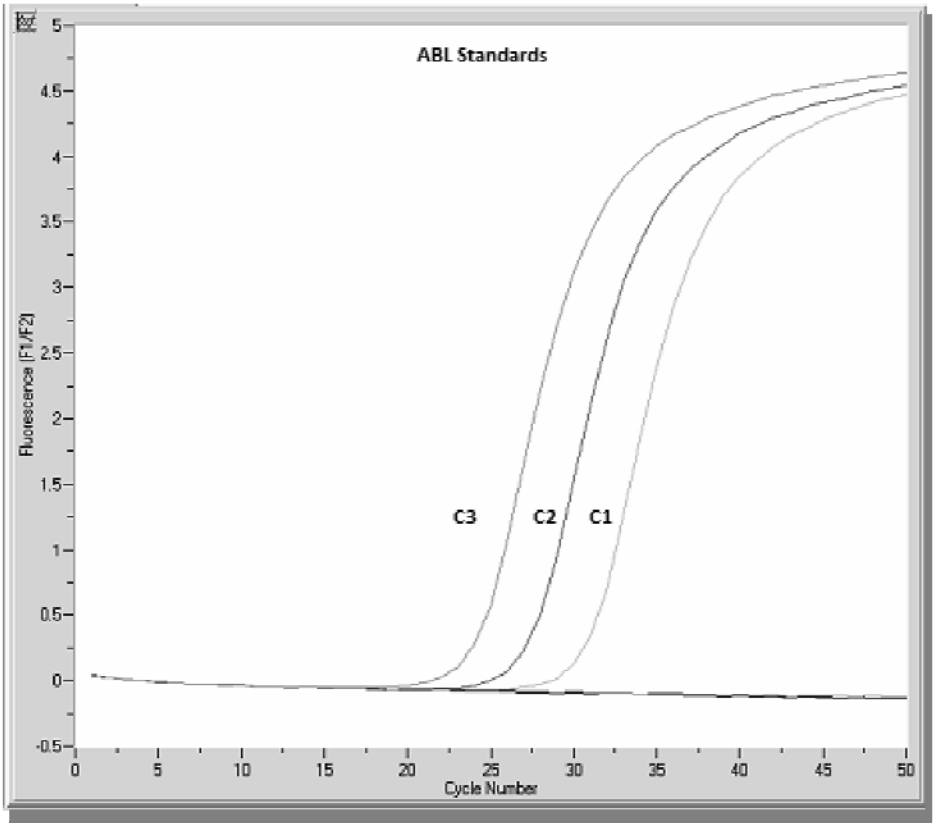

Figure 7. Amplification plot of ABL standards (C1, C2, C3). 10^3, 10^4, and 10^5 copies/5 µl.

As standards are 10-fold dilutions, the theoretical slope of the standard curve is –3.32 for PCR with an optimal efficiency. A slope between –3.0 and -3.9 is acceptable as long as the r^2 is >0.95. However, a value for r^2 of greater than 0.98 is desirable for precise results.

For each gene (ABL and MN1), 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 P1, P2, P3, P4, and P5). The theoretical curve is calculated on 3 and 5 standard dilutions of the standards. A linear regression curve ($y = ax + b$) is calculated for each gene (ABL and MN1), 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.

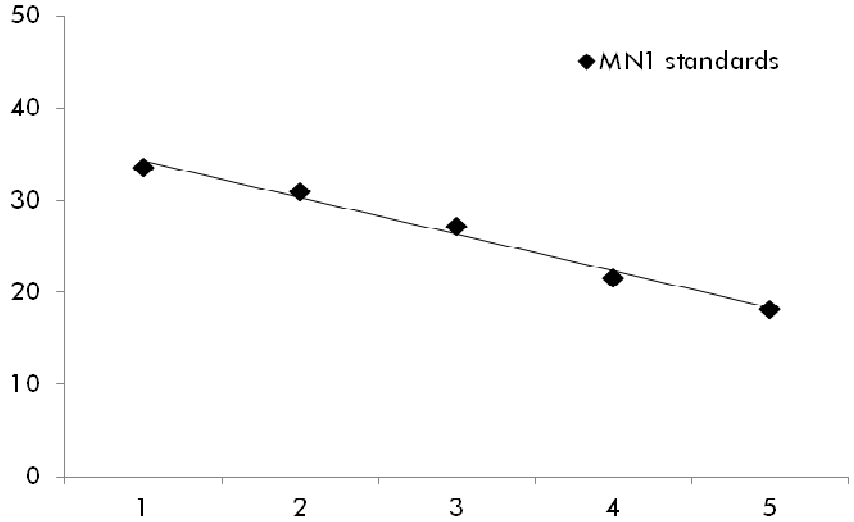

Figure 8. Theoretical standard curve. Measured C_P and C_T are plotted against log10 copy number of each standard.

The respective standard curves are used to determine the MN1 copy number ($MN1_{CN}$) and ABL copy number (ABL_{CN}) for each sample.

Normalized copy number calculation

Results are relative to ~200 ng of total RNA, and corresponding to 5 µl ($1/10^{th}$) of the reverse transcription reaction.

The ratio of the CN values gives the normalized copy number (NCN) per 10,000 copies of ABL:

$$NCN = (MN1_{CN} / ABL_{CN}) \times 10{,}000$$

The NCN result should be calculated for each sample (NCN_{sample}).

Quality control on ABL values

Poor quality of the RNA or difficulties during the qPCR steps may result in a low ABL_{CN}. We recommend discarding results from samples giving ABL_{CN} <10,000.

Water controls

Water control should give zero CN for both ABL and MN1. A positive control results from a cross-contamination. See "Troubleshooting" guide 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 31).

	Comments and suggestions
Negative result for the control gene (ABL) and MN1 in all the samples — standard okay	
a) Poor RNA quality	Always check the RNA quality and concentration before starting.
b) Failure of reverse transcription step	Always check the RNA quality and concentration before starting.
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.
b) Failure of reverse transcription step	Always check the RNA quality and concentration before starting.
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* MN1 Profile*Quant* Kit at –15 to –30°C and keep primers and probe mixes (PPC and PPP) protected from light. See "Reagent Storage and Handling", page 9. 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.
b) Failure of reverse transcription step	Always check the RNA quality and concentration before starting.
Fluorescence intensity too low	
a) Inappropriate storage of kit components	Store the *ipsogen* MN1 Profile*Quant* Kit at –15 to –30°C and keep primers and probe mixes (PPC and PPP) protected from light. See "Reagent Storage and Handling", page 9. Avoid repeated freezing and thawing. Aliquot reagents for storage.

	Comments and suggestions
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 instrument.
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

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Symbols

 <N>	Contains reagents sufficient for <N> reactions
	Use by
	Catalog number
	Lot number
	Material number
	Temperature limitation
	Manufacturer
	Consult instructions for use

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Product	Contents	Cat. no.
ipsogen MN1 Profile*Quant* Kit (24)	For 24 reactions: ABL Control Gene Standards, MN1 Standards, Primer and Probe Mix ABL, Primer and Probe Mix MN1 Gene	676813
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

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Notes

Notes

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