

***ipsogen*[®] FusionQuant[®] Standards**

Control and fusion gene standards for real-time quantitative RT-PCR of fusion gene expression

Recurrent chromosomal translocations result in the expression of specific fusion gene transcripts. These transcripts can be measured by real-time quantitative RT-PCR. Real-time quantitative RT-PCR is performed after reverse transcription of the RNAs, using a pair of specific primers and an internal probe. The *ipsogen* FusionQuant Standards have been validated with EAC (Europe Against Cancer) primers and probes.*

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.

Using 3 or more control gene standards or fusion gene standards with a known number of molecules can establish a standard curve to determine the precise amount of target transcript present in the test sample.

For each experiment, at least one control and one fusion gene transcript are quantified.

Product description: *ipsogen* FusionQuant Control Gene Standards (see Table 1)

- The control gene standards are intended to provide calibration for the quantification of control gene transcripts
- Each control gene standard is comprised of 3–5 tubes containing precisely controlled dilutions of a control gene
- Each control gene standard offers 3–5 dilutions in order to establish standard curves for control genes
- Volume of standard per tube is 50 µl, sufficient for 8 reactions

Product description: *ipsogen* FusionQuant Fusion Gene Standards (see Table 2)

- The fusion gene standards are intended to provide calibration for the quantification of specific fusion gene transcripts
- Each fusion gene standard is comprised of 5 tubes containing precisely controlled dilutions of a given fusion gene
- Each fusion gene standard offers 5 dilutions in order to establish standard curves for fusion genes
- Volume of standard per tube is 50 µl, sufficient for 8 reactions

* Gabert, J. et al. (2003) Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia — A Europe Against Cancer Program. *Leukemia* **17**, 2318.

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



ipsogen FusionQuant Standards

Table 1. ipsogen FusionQuant Control Gene Standards

| Control gene | Cat. no. | Number of tubes | Dilutions (copies in 5 µl) |
|-------------------|----------|-----------------|---|
| TBP | 674091 | 3 | 10 ⁴ , 10 ⁵ , 10 ⁶ |
| B2M | 674191 | 3 | 10 ⁵ , 10 ⁶ , 10 ⁷ |
| SRY3 | 674291 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| β-globine | 674391 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| BCR | 674491 | 3 | 10 ³ , 10 ⁴ , 10 ⁵ |
| GUS | 674591 | 3 | 10 ³ , 10 ⁴ , 10 ⁵ |
| ABL (3 standards) | 674691 | 3 | 10 ³ , 10 ⁴ , 10 ⁵ |
| ABL (4 standards) | 674791 | 4 | 10 ³ , 10 ⁴ , 10 ⁵ , 10 ⁶ |

Table 2. ipsogen FusionQuant Fusion Gene Standards

| Fusion gene | Cat. no. | Number of tubes | Dilutions (copies in 5 µl) |
|----------------------|----------|-----------------|---|
| BCR-ABL1 mbcr | 670291 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| BCR-ABL1 Mbcr | 670391 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| PML-RARA bcr1 | 672191 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| PML-RARA bcr2 | 672291 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| PML-RARA bcr3 | 672391 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| RARA-PML bcr1&2 | 672591 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| RARA-PML bcr3 | 672691 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| TCF3-PBX1 | 674891 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| SIL-TAL | 674991 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| RUNX1-RUNX1T1 | 675091 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| ETV6-RUNX1 | 675191 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| CBFB-MYH11 A | 676091 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| CBFB-MYH11 D | 676191 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| CBFB-MYH11 E | 676291 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| MLL-AF1p e10e2 | 678091 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| MLL-AF4 e10e4 | 678191 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| MLL-AF4 e11e5 | 678291 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| MLL-AF4 e9e5 | 678391 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| MLL-AF6 e8e2 | 678491 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| MLL-AF9 e8e10 | 678591 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| MLL-AF9 type A e10e6 | 678691 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| MLL-AF9 type B e8e9 | 678791 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |

Table 2 continued on next page.

Table 2 continued. *ipsogen FusionQuant Fusion Gene Standards*

| Fusion gene | Cat. no. | Number of tubes | Dilutions (copies in 5 µl) |
|---------------|----------|-----------------|---|
| MLL-DUP e2e8 | 678891 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| MLL-ENL e10e2 | 679091 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| MLL-ENL e9e2 | 679191 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| MLL-ELL e8e2 | 679291 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |
| MLL-ELL e9e2 | 679391 | 5 | 10 ¹ , 10 ² , 10 ³ , 10 ⁵ , 10 ⁶ |

Shipping and Storage

The standards are shipped on dry ice and must be stored at –30°C to –15°C upon receipt. Vortex and centrifuge the tubes before opening.

Warnings and Precautions

When working with chemicals, always wear a suitable laboratory 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

Use of quantitative RT-PCR tests to determine transcript levels requires both the reverse transcription of mRNA and amplification of the generated cDNA by PCR. Therefore, use extreme caution to prevent:

- RNase and DNase contamination
- Carryover contamination by RNA, DNA, control reagents, or PCR resulting in false positive signals

We therefore recommend the following:

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

The *ipsogen FusionQuant Standards* are intended for research use only. Not for use in diagnostic procedures.

ipsogen FusionQuant Standards

Procedure: *ipsogen* FusionQuant Control Gene Standards

To obtain a standard curve to calibrate the control gene in sample RNA, use the corresponding control gene standard with the dedicated primers and probes (extra material not supplied).

We recommend that each dilution of the control gene standard be used in duplicate to establish the standard curve.

Procedure: *ipsogen* FusionQuant Fusion Gene Standards

To obtain a standard curve to calibrate the fusion gene in sample RNA, use the corresponding fusion gene standard with the dedicated primers and probes (extra material not supplied).

We recommend that each dilution of the control gene standard be used in duplicate (10 reactions) to establish the standard curve.

Quality Control

Quality control of the standards has been performed on a LightCycler® 480 instrument. The standards are manufactured according to ISO 13485 standard. Certificates of Analysis are available upon request at www.qiagen.com/support/.

Trademarks: QIAGEN®, FusionQuant®, *ipsogen*®, RNeasy® (QIAGEN Group); Agilent® BioAnalyzer® (Agilent Technologies); LightCycler® (Roche Group); TRIzol® (Molecular Research Center, Inc.).
1095075 03/2015 © 2015 QIAGEN, all rights reserved.

www.qiagen.com
Australia ■ 1-800-243-800
Austria ■ 0800-281011
Belgium ■ 0800-79612
Brazil ■ 0800-557779
Canada ■ 800-572-9613
China ■ 800-988-0325

Denmark ■ 80-885945
Finland ■ 0800-914416
France ■ 01-60-920-930
Germany ■ 02103-29-12000
Hong Kong ■ 800 933 965
India ■ 1-800-102-4114
Ireland ■ 1800 555 049

Italy ■ 800-787980
Japan ■ 03-6890-7300
Korea (South) ■ 080-000-7145
Luxembourg ■ 8002 2076
Mexico ■ 01-800-7742-436
The Netherlands ■ 0800-0229592
Norway ■ 800-18859

Singapore ■ 1800-742-4368
Spain ■ 91-630-7050
Sweden ■ 020-790282
Switzerland ■ 055-254-22-11
Taiwan ■ 0080-665-1947
UK ■ 0808-2343665
USA ■ 800-426-8157

