# One-Step Detection Kits for Fusion Transcripts

## Real-Time PCR assay

- Detects and quantifies PML-RARA fusion transcripts
- One-step cDNA synthesis
- 24 tests per kit
- Available as RUO (US) / IVD (EU)

## INTENDED USE:

The PML-RARA One-Step Detection Kit is intended for the relative quantitative detection of three fusion transcripts t(15;17)(q24;q21) PML-RARA bcr1, bcr2, and bcr3 in bone marrow or peripheral blood samples.
This kit is intended for research use only. Not for use in diagnostic procedures.

## FOR USE WITH:

- Applied Biosystems - 7500/7500 Fast, StepOne Plus
- Bio-Rad - CFX96
- Qiagen - RotorGene® 3000, 6000
- Roche - LightCycler® 480, Cobas® 4800, Cobas® z480

## PACKAGE CONTENTS

This product contains the following materials sufficient for 24 reactions, including samples and controls.

| Description | Tubes |
|---|---|
| One-Step RT-qPCR Reaction Mix (2X) | 1 |
| RT Enzyme Mix (20x) | 1 |
| Molecular Grade Water | 1 |
| PML-RARA (bcr1) Detection Primer/Probe Mix | 1 |
| PML-RARA (bcr2) Detection Primer/Probe Mix | 1 |
| PML-RARA (bcr3) Detection Primer/Probe Mix | 1 |
| Fusion Transcript Positive Control | 1 |

## HANDLING AND STORAGE

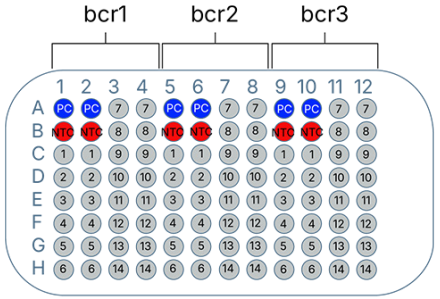
This product is shipped on frozen ice packs and may be thawed upon arrival. Do not ship the product on dry ice. The contents of the shipment should be frozen at -25°C to -15°C immediately upon receipt. Do not store the kit or any of its components below -25°C as it will adversely affect the performance of the assay.

- Store all unopened components in original containers.
- Centrifuge the tubes before opening.
- The expiration date of each component is printed on each tube label. This product will maintain its performance through this date. Its performance is not guaranteed after the expiration date.

Storage and handling of the components of this product under conditions other than those described above may affect the performance of this assay and adversely affect the results.

## PLATE SETUP

The assay requires 3 reactions in duplicate per sample. The recommended plate layout is shown above. A single 96-well PCR plate can accommodate up to 14 unknown samples, one positive control mix and one no template control (NTC) for each one of the 3 primer mixes.




## PRINCIPLE OF THE ASSAY

Chromosomal translocations result in creation of gene fusion transcripts that aberrantly modulate various cellular processes. The table below lists the 3 main PML/RARA t(15;17) translocations that are commonly associated with acute promyelocytic leukemia (APL).

| Translocation | Fusion Gene | Diagnosis | Frequency |
|---|---|---|---|
| t(15;17)(q24;q21) | PML-RARA bcr1 | APL | 55% |
| t(15;17)(q24;q21) | PML-RARA bcr2 | APL | 5% |
| t(15;17)(q24;q21) | PML-RARA bcr3 | APL | 40% |

Use the PML-RARA One-Step Detection kit to screen and monitor residual or recurrent APL.
This kit utilizes primer sets and a FAM-labeled hydrolysis probe to detect and quantify the presence of the specified fusion transcript. The primer mix also contains primers and a Cal Fluor Orange 560 (VIC equivalent)- labeled probe that detect the amplification of ABL as an internal control. This enables the kit to use the comparative Ct method (Pfaffl method) to determine the target-reference gene ratios without the need to run control standards of known copy number. This kit has been formulated for highly reproducible first-strand cDNA synthesis and subsequent real-time PCR in a single tube.

# ASSAY PERFORMANCE

## *Precision*

The assay's reproducibility was determined by analyzing synthetic controls representing PML-RARA Bcr 1, 2 and 3 tested in three ten-fold serial dilution (11.7 – 1170 copies/reaction) in the background of wildtype RNA. Samples were tested in 12 repeats across five reagent lots, by two different operators, on non-consecutive days. This study was performed on Applied Biosystems® 7500 Fast, software version 2.0

### *PML-RARA (bcr1):*

| Sample Name | Average VIC Ct | SD | Average Target Ct | SD |
|---|---|---|---|---|
| PC | 19.52 | 0.12 | 24.11 | 0.21 |
| Bcr1 Fusion Gene Ratio 1 (1170 copies) | 22.27 | 0.88 | 28.02 | 1.04 |
| Bcr1 Fusion Gene Ratio 0.1 (117 copies) | 21.43 | 0.69 | 31.49 | 0.84 |
| Bcr1 Fusion Gene Ratio 0.01 (11.7 copies) | 21.43 | 0.72 | 34.36 | 1.01 |

### *PML-RARA (bcr2):*

| Sample Name | Average VIC Ct | SD | Average Target Ct | SD |
|---|---|---|---|---|
| PC | 19.59 | 0.12 | 23.72 | 0.22 |
| Bcr2 Fusion Gene Ratio 1 (1170 copies) | 21.6 | 0.72 | 27.88 | 0.54 |
| Bcr2 Fusion Gene Ratio 0.1 (117 copies) | 21.49 | 0.73 | 31.38 | 0.4 |
| Bcr2 Fusion Gene Ratio 0.01 (11.7 copies) | 21.48 | 0.65 | 34.37 | 0.47 |

### *PML-RARA (bcr3):*

| Sample Name | Average VIC Ct | SD | Average Target Ct | SD |
|---|---|---|---|---|
| PC | 19.61 | 0.14 | 24.00 | 0.26 |
| Bcr3 Fusion Gene Ratio 1 (1170 copies) | 21.63 | 0.72 | 28.43 | 0.62 |
| Bcr3 Fusion Gene Ratio 0.1 (117 copies) | 21.50 | 0.66 | 31.81 | 0.61 |
| Bcr3 Fusion Gene Ratio 0.01 (11.7 copies) | 21.46 | 0.66 | 35.06 | 1.00 |

## *Linearity*

Bcr 1, 2 and 3 synthetic controls in the background of wildtype RNA were tested in four ten-fold dilutions, in duplicate on Applied Biosystems® 7500 Fast, software version 2.0. Ct values were plotted against the $Log_{10}$ of the target input, and reaction efficiency was calculated as follows:

Efficiency = 10^(-1/slope) – 1

| | | Average Ct | | |
|---|---|---|---|---|
| Input (Copies) | Input Log | Bcr 1 | Bcr 2 | Bcr 3 |
| 11700 | 4.07 | 23.85 | 24.00 | 23.94 |
| 1170 | 3.07 | 27.40 | 27.64 | 27.57 |
| 117 | 2.07 | 30.83 | 30.88 | 30.96 |
| 11.7 | 1.07 | 33.82 | 34.47 | 33.98 |
| **Reaction Efficiency** | | **100%** | **94%** | **99%** |

## *Limit of Detection*

Synthetic controls representing PML-RARA Bcr 1, 2 or 3 were diluted in the background of wildtype RNA at the minimum (VIC of ~21.5) and maximum (VIC Ct of ~ 18.5) reaction input. Samples were tested in 60 replicates per reaction, with 3 reagent lots, on Applied Biosystems® 7500 Fast, software version 2.0. The results demonstrate high sensitivity of 4 target copies per reaction, at ≥95% confidence.

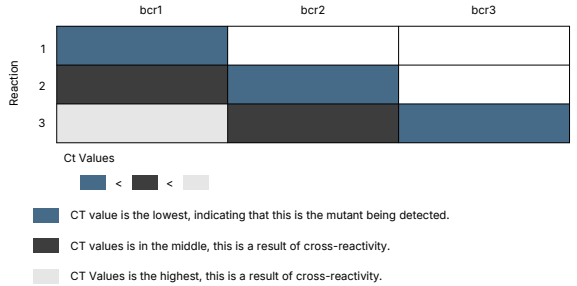
| | | Max Input | | | | Min Input | | | |
|---|---|---|---|---|---|---|---|---|---|
| Reaction | Copies per Reaction | Average VIC Ct | SD | Average FAM Ct | SD | Average VIC Ct | SD | Average FAM Ct | SD |
| bcr1 | 4 | 18.54 | 0.17 | 36.54 | 0.63 | 21.46 | 0.23 | 37.00 | 0.77 |
| bcr2 | 4 | 18.47 | 0.17 | 36.38 | 0.82 | 21.39 | 0.31 | 36.10 | 0.76 |
| bcr3 | 4 | 18.57 | 0.19 | 36.19 | 0.73 | 21.45 | 0.27 | 36.19 | 1.08 |

## *Limit of Blank*

Limit of Blank of each reaction was tested using four different wildtype RNA samples at 8 inputs ranging between 125 and 4000 ng per well (corresponding to a VIC Ct of 22 to 17). Each input was tested in 4 to 6 repeats. In addition, molecular grade water was tested in 10 repeats. Samples were tested across 2 reagent lots, on Applied Biosystems® 7500 Fast, software version 2.0. No non-specific amplification was detected under the assay's analytical cutoff.

## *Cross Reactivity*

Cross-reactivity of bcr1, 2 and 3 primers was demonstrated using Invivoscribe IVS-0020 clonal control RNA for PML-RARA translocation t(15;17)(q24;q21). RNA control sample was tested at 800 ng/well (VIC Ct of 19.7±0.13). The assay was performed in duplicate on an ABI7500 Fast Instrument across 3 reagent lots.




Bcr1, 2 and 3 variants of the PML-RARA fusion are the result of a breakpoint in intron 2 of RARA and one of three breakpoints in PML (intron 6, exon 6 or intron 3). The three fusion variants are detected and amplified by the One-step Detection kit using specific primers and probes located around the expected PML-RARA fusion region. Since the primers used in the detection of the different fusions are located in tandem within the PML gene, based on the location of the breakpoint, all 3 primers located distal to the fusion breakpoint may produce amplicons, resulting with the lowest Ct value in the target reaction (near the breakpoint), and higher Ct values from amplification of longer amplicons (far from the breakpoint).

**bcr1 positive sample:**
In a sample that is positive for bcr1 the resulting amplification would have the lowest Ct value for bcr1, followed by amplification for bcr2, then by amplification for bcr3. The signal for bcr1 is specific and the signals for bcr2 and bcr3 are seen as a result of cross reactivity. For samples with low copy bcr1 fusion variant, the bcr2 and bcr3 cross-reactive amplification are shifted and may not produce a Ct value.

**bcr2 positive sample:**
In a sample that is positive for bcr2 the resulting amplification would have the lowest Ct value for bcr2, followed by amplification for bcr3. The bcr1 primers do not hybridize with bcr2 fusion variant due to missing exon, therefore the bcr1 primer set does not cross-react with a bcr2 positive sample. For samples with low copy bcr2 fusion variant, the bcr3 cross-reactive amplification is shifted and may not produce a Ct value.

**bcr3 positive sample:**
In a sample that is positive for bcr3 the resulting amplification would produce a Ct value for bcr3. The bcr1 and bcr2 primers do not hybridize with the bcr3 fusion variant due to missing exons, therefore the bcr1 and bcr2 primer sets do not cross-react with a bcr3 positive sample.

# CONTACT

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For research use (RUO) in the U.S
For in vitro diagnostic use (IVD) in the European Union