BCR-ABL REAL TIME QUANTITATIVE PCR DETECTION KIT

ONKOTEST RQ2002-20

onkotest

Product Information

The Philadelphia (Ph) chromosome observed in chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL) is formed by the reciprocal translocation between chromosome 9 and 22 - specifically designated as t(9;22)(q34;q11). This translocation creates an elongated chromosome 9 and a truncated chromosome 22 known as the Philadelphia chromosome; and results in the juxtaposition of the 5' end of the BCR gene and the 3' end of the ABL gene; generating a BCR-ABL chimeric oncogene. Different breakpoints will result in different oncogenic products differing in size. Nearly 99% of CML patients, 20-40% of adult ALL and 2-5% of pediatric ALL patients are positive for the Ph chromosome at diagnosis. Because of different breakpoints in the BCR and ABL genes, the oncoproteins coded by BCR-ABL are disease specific. The 190 KDa BCR-ABL product is observed in 60% of adult Ph positive ALL patients and in 80% of Ph positive pediatric ALL patients. The fusion between BCR exon 1 (e1) which is within the m-BCR region and ABL exon 2 (a2) results in the e1a2 chimeric gene that encodes for a 190kDa oncoprotein observed in a subset of ALL patients (Figure 1).

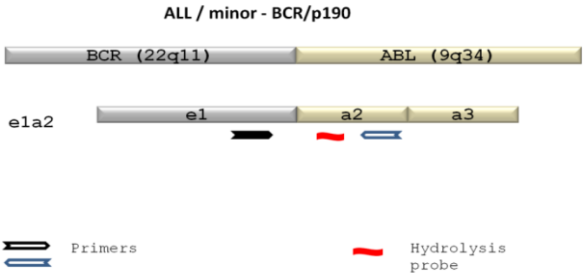

Figure 1: Annealing locations of the PCR primers and hydrolysis probe

The **Onkotest RQ2002-20** kit allow to detect quantification of BCR-ABL copy number by real-time quantitative PCR (qPCR) technique.

The **Onkotest RQ2002-20** kit uses fluorescent labeled probe and PCR primers to detect the BCR-ABL encoding the chimeric gene product mRNA with high sensitivity. The main working principle is to use known amounts of BCR-ABL and ABL (internal control gene) calibrators to generate a standard curve from which sample BCR-ABL mRNA can be quantified relative to control ABL gene expression. cDNA converted from total RNA of patient's bone marrow/blood samples are used as template in the reactions (reagents for cDNA conversion are not provided with the kit). Sample cDNA quality is checked by using a probe and PCR primers of an internal control gene (ABL), provided with the kit. The **Onkotest RQ2002-20** kit will only detect BCR-ABL mRNA that is specific for the primers used; it kit will not detect other mRNA breakpoint products that are outside the sequence confined by the PCR primers provided within the kit. **The Onkotest RQ2002-20** kit is designed to work with all real time thermal cycler instruments.

Kit Contents

Tube No:	Labeling&Contents	Volume
1	BCR-ABL Primer Mix	110 µl
2	BCR-ABL Probe	110 µl
3	ABL Primer Mix	90 µl
4	ABL Probe	90 µl
5	PCR-Grade dH_2O	500 µl
	Content-Calibrators	**Volume**
	ABL 10e2	50 µl
	ABL 10e3	50 µl
	ABL 10e4	50 µl

	ABL 10e5	50 µl
	BCR-ABL 10e1	50 µl
	BCR-ABL 10e2	50 µl
	BCR-ABL 10e3	50 µl
	BCR-ABL 10e4	50 µl
	BCR-ABL 10e5	50 µl
	BCR-ABL 10e6	50 µl

- **All reagents are ready to use**

Kit Description

The **Onkotest RQ2002-20** kit uses cDNA of patients (converted from total RNA by reverse transcription) as template for PCR reactions. Real time PCR will amplify the target BCR-ABL and ABL regions by using highly specific primers provided within the kit. The hydrolysis probe will specifically hybridize to its own complementary target sequence within the amplified PCR product. It is a dual labeled probe in which the fluorophore FAM (6-carboxyfluorescein) is covalently attached to the 5'-end; while a quencher, BHQ (Black hole quencher) is attached at the 3'-end. The quencher molecule quenches the fluorescence emitted by the fluorophore, so as long as the fluorophore and the quencher are in proximity, fluorescence signals are inhibited. When the probe hybridizes to its specific sequence, the 5´–>3´ exonuclease activity of the polymerase degrades the probe and breaks the close proximity between the fluorophore and the quencher. As a result the fluorophore is relieved of the quenching effect and is able to emit fluorescence which can be detected (Figure 2). The fluorescence detected is directly proportional to the amount of fluorophore released, therefore also directly proportional to the

amount of DNA template present in the PCR. The hydrolysis probe is both highly sensitive and highly specific; for it requires the presence and amplification of its complementary sequence by the specific primers provided within **Onkotest RQ2002-20** kit.

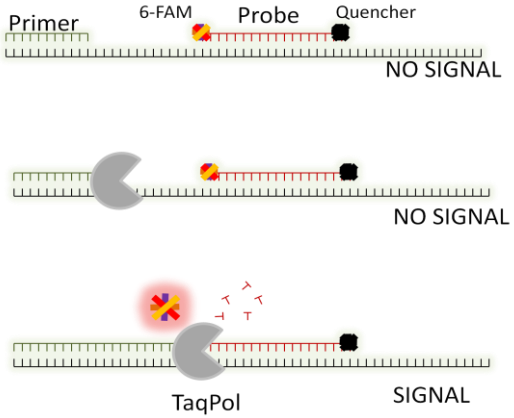

Figure 2: Hydrolysis probe

The positive and internal controls provided with the kit are aimed to prevent false negative results that may be the outcome of failed reactions or poor template quality. Using H_2O as a negative control aims to prevent false positive results by the detection of cross contamination

Sample Material

The **Onkotest RQ2002-20** kit is optimized for the accurate detection of BCR-ABL transcripts in bone marrow and/or peripheral blood samples of patients. The kit uses cDNA (converted from total RNA by reverse transcription) as template for the PCR reactions. Using patient RNA directly as template will not yield results.

**EDTA-ethylenediaminetetraacetic acid is preferred as anticoagulant, heparin may inhibit PCR reactions.*

IMPORTANT: To minimize cross contamination risks, the calibrator tubes inside the kit should be stored separately from test samples and added to the reaction mix in a separate area during assay preparation.

Number of Tests

The **Onkotest RQ2002-20** kit contains primers and probes for BCR-ABL and internal control ABL reactions, enough for duplicate analyses of 20 patient samples. Calibrators and patient samples should be work as duplicate.

Handling & Storage

The components of kit should be stored at -20°C. Protect fluorescent probes (tubes **2** and **4**) from light. While setting up PCR reactions, kit components should be placed on ice**. Maximum of freeze-thaw cycles:5**

Materials Required But Not Provided

Equipment

Real-time thermal cycler
Laminar flow hood/biological safety cabinet
Vortex
Spin-down microcentrifuge
Micropipettes

Consumables

Real Time PCR master mix

cDNA conversion kit
Sterile filtered pipette tips
Serological pipettes
0,2 ml PCR tubes/Capillary tubes/strip tubes/plates (depending on real-time instrument)

Important Notes & Precautions:

- Separate areas dedicated for RNA isolation, cDNA/PCR reaction preparation and agarose gel electrophoresis is strongly recommended.
- Lab coats and safety equipment (goggles ect.) should be specifically designated to each area
- Separate micropipette sets for each area is strongly recommended.
- Micropipette tips should be DNAse-RNAse free and preferably filtered.
- To prevent cross contamination between patient samples, total RNA isolation of samples should be performed separately.
- Avoid biohazard exposure by observing universal precautions when handling all biological materials.
- Minimize cross contamination by storing calibrator tubes separately from test samples and add to the reaction mix in a separate area during assay preparation.
- Avoid direct contact of reagents. In case of direct contact, wash *thoroughly* with water. Seek medical care in case of inhalation and/or swallowing.
- Do not use reagents with different lot numbers in sample reactions.

Before You Begin

Before starting, be sure the tube contents are fully dissolved. Mix tube contents briefly by vortexing, followed by a spin-down centrifugation to bring down tube contents. Keep all test samples and kit contents on ice when in use.

Procedure

Negative control (NC) - (dH2O): The "no template negative control". This control replaces template cDNA with water in the PCR reactions. It aims for the detection of any cross contamination (false positive results). The negative control should not give any positive signal. In cases where a positive reaction signal is detected, all reactions must be repeated.

Patient sample internal control (IC-ABL): The ABL gene product is used as internal control. cDNA from patient samples are used as template for this reaction. The PCR product size is 123 bp. It aims for the detection of poor sample quality (false negative results). Patient sample PCRs that are negative for ABL, designate poor template quality (RNA and/or cDNA unsuitable for analyses). In case of a negative internal control (negative result for ABL expression), a new cDNA conversion from total RNA should be performed and both BCR-ABL and ABL PCR reactions should be repeated. New RNA extraction from patient bone marrow/peripheral blood should be performed in cases where a second negative result for the internal control is obtained.

Preparation of the Real-time PCR Mix

PCR Reaction Panel for One Patient *

	Negative Control (ABL)*	Calibrator (ABL)	Negative Control (BCR-ABL)*	Calibrator (BCR-ABL)	Patient ABL	Patient BCR-ABL
ddH_2O (tube 5)	to 20µl	to 20µl	to 20µl	to 20µl	to 20µl	to 20µl
Real -time master mix (not provided)	1X	1X	1X	1X	1X	1X
BCR-ABL primer mix (tube 1)	-	-	1 µl	1 µl	-	1 µl
BCR-ABL probe (tube 2)	-	-	1 µl	1 µl	-	1 µl
Calibrators (ABL/BCR-ABL)	-	5 µl	-	5 µl	-	-
ABL primer (tube 3)	1 µl	1 µl	-	-	1 µl	-
ABL probe (tube 4)	1 µl	1 µl	-	-	1 µl	-
Patient cDNA sample	-	-	-	-	5 µl	5 µl

Total reaction volume 20 µL

*** Negative Control: ddH_2O used instead of template**

Thermal Profile for Real-Time PCR Instrument

Use the following profile on your real-time PCR instrument. When creating your thermal profile if your instrument requires you to define parameters for the 5' and 3' ends: select 6-FAM for the 5 prime end, and "quencher dye" for the 3 prime end.

Denaturation	95˚C	10 minutes	1 cycle
Reaction	95˚C	10 seconds	45 cycles
	58˚C	40 seconds (signal accumulation occurs)	
Cooling	40 °C	1 minute	1 cycle

Analyses

The instrument you use will evaluate the real-time PCR reactions using its own software by generating standard curves for both BCR-ABL and ABL; from which relative amounts of the genes can be calculated in both control and test samples. By dividing the BCR-ABL value with the ABL value we obtain the BCR-ABL/ABL.

Evaluation of Results

We recommend reporting patient BCR-ABL copy number by the following calculation:

Normalized BCR-ABL $_{\text{copy number}}$= BCR-ABL /ABL

Product Specifications

Kit Capacity	20 samples duplicate
Control Gene	ABL
Reported Values	BCR-ABL Transcript
Components	BCR-ABL primer mix/probe ABL primer mix/probe DNA Plasmid Control (BCR-ABL)
Tested Platforms	Roche® LC480, LightCycler® 1.5, 2.0; ABI® StepOnePlus™; Corbett® Rotor-Gene® 6000 and Rotor-Gene Q
Product Order No.	RQ2002-20

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The following symbols may appear on the packaging and labeling:

CE	**CE sign**
	Manufacturer
	Consult instructions for use
IVD	**In vitro diagnostic**
	Manufacturing date
REF	**Catalog number**
LOT	**Lot number**
-22°C -18°C	**Temperature limitation**
	Expiration date

KK2002-20/01.03.2016/00 CE