

BCR-ABL (p190) REAL TIME PCR DETECTION KIT ONKOTEST R2001-20



Keep the kit at -15°C to -25°C

Rev. 1.3

Product Information

www.onkotech.com

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 R2001-20 kit uses fluorescent labelled probe and PCR primers to detect the BCR-ABL mRNA encoding the chimeric gene product p190 with high sensitivity. cDNA converted from total RNA of ALL patients is used as template in the reactions. cDNA quality is checked by using a probe and PCR primers of an internal control gene (ABL), provided with the kit. The positive control template -also provided with the kit- will verify that the PCR reactions work.

The **onkotest R2001-20** kit will only detect the BCR-ABL mRNA that gives the 190 kDa(p190) protein product, specific for ALL. This kit will not detect p210, p230 and other mRNA breakpoint products that are outside the sequence confined by the PCR primers provided within the kit.

Kit Contents

Tube	Labeling	Volume& Content
1	Real Time Master Mix (2X)	565 µl (2X) - enough for 56 reactions. Ready to use real time PCR reaction mix.
2	BCR-ABL Primer Mix	40 µl- enough for 20 patient samples, 8 positive control and 8 negative control reactions.
3	BCR-ABL probe	40 µl- enough for 20 patient samples, 8 positive control and 8 negative control reactions.
4	Positive Control	40 µl- contains a positive control template enough for 8 reactions.
5	ABL Primer Mix	20 µl- enough for real time reactions of 20 patient samples.
6	ABL Probe	20 µl- enough for real time reactions of 20 patient samples.
7	H ₂ O (PCR grade)	500 µl- Nuclease free. PCR grade. Use for reaction mixture preparation and as template for negative control reactions.
8	ROX (6µM)	40 µl

The onkotest R2001-20 kit is designed to work with all real time thermal cycler instruments (the onkotest R2001-20 kit has been optimized to work without ROX dye, it can easily be used with ABI real time instruments without ROX. Nevertheless if ROX is to be used, add 1 µl to each reaction tube (the final concentration will be 300nM after the addition of 1 µl ROX). If ROX is added, do not forget to also reduce dH₂O by 1 µl to keep the total reaction volume at 20 µL)

Kit Description

The **onkotest R2001-20** kit uses cDNA of ALL patients (converted from total RNA by reverse transcription) as template for PCR reactions. Real time PCR will amplify target the BCR-ABL and ABL regions by using highly specific primers provided with 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 R2001-20** kit.

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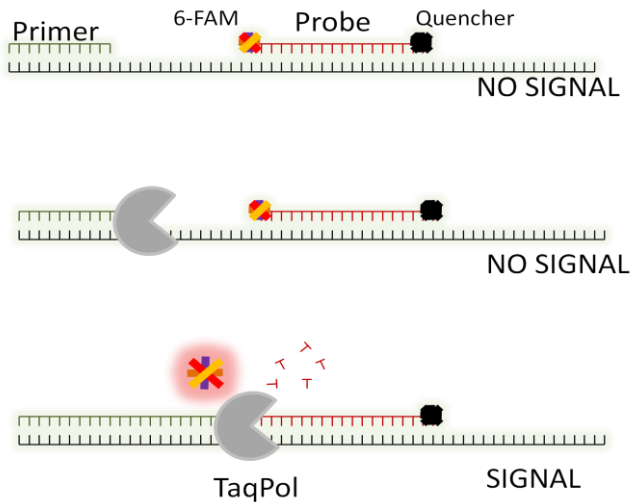


Figure 2: Hydrolysis probe

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 H₂O as a negative control aims to prevent false positive results by the detection of cross contamination.

Sensitivity:

The **onkotest R2001-20** kit is able to detect 250 copies of BCR-ABL oncogene mRNA in 2 micrograms of total RNA isolated from BCR-ABL positive cells.

Sample Material:

The **onkotest R2001-20** kit is intended for the accurate detection of BCR-ABL (p190) transcripts in bone marrow or peripheral blood samples (EDTA - ethylenediaminetetraacetic acid is the preferred as anticoagulant, heparin may inhibit the PCR reaction) of CML 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.

Number of Tests:

The **onkotest R2001-20** kit contains the reaction mix, primers and probes enough for BCR-ABL and internal control ABL reactions of 20 patient samples. The kit also provides the reaction mix, primers and probes enough for 8 positive control and 8 negative control reactions.

Handling & Storage

The components of kit should be stored at -20°C . Protect fluorescent probes (tubes 3 and 6) from light. While setting up PCR reactions, kit components should be placed on ice. Multiple freeze-thaw cycles may result in the degradation of protein reagents.

IMPORTANT: In order to prevent cross contamination, the positive control tube inside the kit should be stored separately from test samples and added to the reaction mix in a separate area during assay preparation. **Materials Required But Not Provided**

Equipment

Real-time thermal cycler

Laminar flow hood/biological safety cabinet

Vortex

Spin-down microcentrifuge

Micropipettes

Consumables

Sterile filtered pipette tips

Serological pipettes

0,2 ml PCR tubes/Capillary tubes/strip tubes/plates (depending on the 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.
- In order 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.

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): Tube number 7 is 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. The negative control should not give any positive signal. In cases where a positive reaction signal is detected, **ALL** reactions must be repeated. The kit provides BCR-ABL primers and probe enough for 8 reactions.

Positive control (PC): Tube number 4 contains positive control template. Bcr-Abl detection **MUST BE** positive when this control is used. Otherwise all reactions for all samples must be repeated. The positive control template has been derived from a source encoding the BCR-ABL, e1a2 chimeric gene. It gives a band 90 bp in size on agarose gel electrophoresis. To avoid false positive results resulting from cross contamination we strongly suggest the addition of positive control cDNA in to its reaction mix in a separate area. The kit provides positive control cDNA enough for 8 reactions. This positive control template should not be diluted when used. We recommend the positive control to be evaluated after the 13th cycle. Signal observed at lower cycle numbers should be evaluated as false positive.

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. 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

Real-time PCR Reaction Panel for One Patient *:

	Negative Control **	Positive Control	Patient ABL	Patient BCR-ABL
dH ₂ O (tube 7)	8 µl	3 µl	3 µl	3 µl
Real time master mix (2X)(tube 1)	10 µl	10 µl	10 µl	10 µl
BCR-ABL primer mix (tube 2)	1 µl	1 µl	-	1 µl
BCR-ABL probe (tube 3)	1 µl	1 µl	-	1 µl
Positive Control (tube 4)	-	5 µl	-	-
ABL primer (tube 5)	-	-	1 µl	-
ABL probe (tube 6)	-	-	1 µl	-
Patient cDNA template	-	-	5 µl	5 µl

Total reaction volume 20 µL

* When more than one patient sample is being tested in a single reaction panel, using one positive and one negative reaction control will be sufficient.

**dH₂O replaces the template

Thermal Profile for Real-Time PCR Instrument

Denaturation	95°C	10 minutes	1 cycle
Reaction	95°C	10 seconds	35 cycle
	58°C	40 seconds (signal acquisition step)*	
Cooling	40 °C	1 minute	1 cycle

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.

Evaluation:

The instrument you use will evaluate the real-time PCR reactions using its own software. The onkotest R2001-20 kit has been optimized to perform BCR-ABL gene product analyses with very high sensitivity. PCR primers and probes have been design to prevent non-specific reactions. Nevertheless we recommend running the PCR products on 2% agarose gel electrophoresis and visualizing the expected specific band. Patients with e1a2 type BCR-ABL chimerism are expected to be positive for a 90 bp size product. The expected band size for ABL is 123 bp (Figure 4).

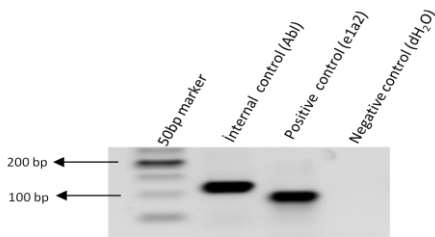









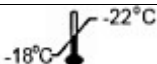

Figure 4: Agarose gel electrophoresis of PCR control reactions.

Product Specifications

Kit Capacity	20 samples
Control Gene	ABL
Reported Values	BCR-ABL Transcript (e1a2)
Components	BCR-ABL primer mix/probe ABL primer mix/probe DNA Plasmid Control (BCR-ABL)
Dynamic Range/Sensitivity	5 pg RNA
Tested Platforms	Roche® LC480, LightCycler 1,5, 2,0; ABI StepOnePlus™; Corbett® Rotor Gene® 6000
Product Order No.	R2001-20

Symbols

The following symbols may appear on the packaging and labeling:

	CE sign
	Manufacturer
	Consult instructions for use
	In vitro diagnostic
	Manufacturing date
	Catalog number
	Lot number
	Temperature limitation
	Expiration date



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