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BCR-ABL Suggested Protocol

BCR-ABL Clinical Relevance:

The BCR-ABL fusion results in constitutive ABL tyrosine kinase activity contributing to unregulated cell division. The BCR-ABL fusion is found in 95% of chronic myelogenous leukemia (CML), 25–30% adult acute lymphoblastic leukemia (ALL), and 2–10% child ALL. Researchers have identified the utility of measuring BCR-ABL transcripts to aid in the assessment of minimal residual disease (MRD) and the response to treatment. An international consortium has developed "International Standards" allowing the comparison of BCR-ABL levels measured by different quantitative PCR assays.[1,2,3]

Scope of Procedure:

The following is a recommended procedure for the detection of the three most common BCR-ABL fusions by quantitative PCR (b2a2, b3a2, and e1a2). Researchers are advised to utilize this protocol as a guide in the development of their own procedure. This protocol was developed utilizing ancillary reagents and equipment purchased from external vendors. Protocol modifications will be required dependent on the selection of ancillary reagents and equipment utilized in your laboratory.

The following protocol provides instructions for processing samples. Laboratories are encouraged to develop their own guidelines on the selection and use of standards, controls and data analysis.

CytoGenes Reagents (Required):

Cat #	Item
BA-WT	BCR-ABL Wt Primer Mix
BA-B2	BCR-ABL b2a2 Primer Mix
BA-B3	BCR-ABL b3a2 Primer Mix
BA-E1	BCR-ABL e1a2 Primer Mix

CytoGenes Reagents (Optional):

Cat #	Item	Assay Targets Included	Concentration
BA-S1	BCR-ABL Standard-1	ABL, BCR-ABL (b2a2, b3a2, e1a2)	$5e^5$ copies/ul
BA-S2	BCR-ABL Standard-2	ABL, BCR-ABL (b2a2, b3a2, e1a2)	$5e^3$ copies/ul
BA-S3	BCR-ABL Standard-3	ABL, BCR-ABL (b2a2, b3a2, e1a2)	50 copies/ul
BA-HC	BCR-ABL High Control	ABL, BCR-ABL (b2a2, b3a2, e1a2)	3ng/ul
BA-LC	BCR-ABL Low Control	ABL, BCR-ABL (b2a2, b3a2, e1a2)	0.3ng/ul

Ancillary Reagents (Recommended):

- EXPRESS One-Step Superscript® qRT-PCR (ThermoFisher)

Equipment and Supplies (Required):

- Quantitative PCR instrument
- PCR plates, tubes and sealing film.
- Pipettors (range 1-1000ul)
- Aerosol barrier pipette tips.
- Microcentrifuge

Protocol:

1. Isolate RNA from samples utilizing standard procedures utilized by your laboratory.

2. Prepare separate PCR reactions for each of the PCR primer sets to be tested. (Note: for multiple samples, prepare a master mix and scale the volumes appropriately based on the number of samples to be tested).

Reagent	Volume (ul)
EXPRESS SuperScript® qPCR SuperMix	10
CytoGenes 25X PCR Primer Mix	0.8
EXPRESS SuperScript® Mix for One-Step qPCR	2
Nuclease Free Water	5.2
Sample	2
Total volume	20

3. Prior to initiating the run, program Q-PCR instrument to detect the probes utilizing the appropriate filter sets. The probes in each of the master mixes are labeled with a FAM reporter and BHQ quencher.

4. Initiate PCR protocol appropriate for a one-step reverse transcriptase Q-PCR reaction. The following is a recommended protocol based on our instruments and ancillary reagents utilized.

Cycle	Step	Temp (°C)	Time (Min:Sec)	Repeats
1	1	60	15:00	1
2	1	95	2:00	1
3	1	95	:20	40
	2	60	:30	

5. Analyze results.

References:

1. White HE, Matejtschuk P, Rigsby P, Gabert J, Lin F, Lynn Wang Y, Branford S, Müller MC, Beaufils N, Beillard E, Colomer D, Dvorakova D, Ehrencrona H, Goh HG, El Housni H, Jones D, Kairisto V, Kamel-Reid S, Kim DW, Langabeer S, Ma ES, Press RD, Romeo G, Wang L, Zoi K, Hughes T, Saglio G, Hochhaus A, Goldman JM, Metcalfe P, Cross NC. Establishment of the first World Health Organization International Genetic Reference Panel for quantitation of BCR-ABL mRNA. Blood. 2010 Nov 25;116(22):e111-7. Epub 2010 Aug 18.

2. WHO International Standard. 1st WHO International Genetic Reference Panel for quantitation of BCR-ABL translocation by RQ-PCR.

3. Hughes T, et al. Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: Review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. Blood. 2006;108:28-37.