mSEP™ BCR::ABL1 Major dPCR Test Instructions For Use

For Research Use Only

REF	MBCR-RA192
Σ	192
	Store between -30°C to -10°C
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IFU-BCR-RA01-1000

Contents

Intended Use

mSEP™ BCR::ABL1 Major dPCR Test is a one-step Reverse Transcription digital Polymerase Chain Reaction (RT-dPCR) for quantifying BCR::ABL1 fusion and ABL1 transcript in total RNA from peripheral blood of diagnosed t(9;22) Chronic Myeloid Leukemia (CML) patient expressing p210 Major BCR::ABL1 fusion transcript type b2a2 (e13a2) and/or b3a2 (e14a2).

It is intended to measure BCR::ABL1 fusion to endogenous control ABL1 gene. The result is reported as a percent reduction from a baseline of 100% on the International Scale (% I.S.) and on a log Molecular Reduction (MR) scale in t(9;22) positive CML patient for minimal residual disease monitoring during treatment with Tyrosine Kinase Inhibitor.

This test does not differentiate between b2a2 (e13a2) and b3a2 (e14a2) fusion transcript, and does not monitor other rare fusion transcripts resulting from t(9;22).

This test is not intended for the diagnosis of CML and other atypical fusion transcripts.

Testing with mSEP™ BCR::ABL1 Major dPCR Test is intended for use by trained laboratory professionals who are proficient in performing RT-dPCR assay on the QuantStudio™ Absolute Q™ Digital PCR System.

Summary and Explanation

More than 90% of Chronic Myeloid Leukemia (CML) cases is characterized by the presence of Philadelphia chromosome, which is the product of a reciprocal translocation between the long arms of chromosomes 9 and 22, t(9;22). The breakpoint cluster region of BCR is located in chromosome 22 and the ABL1 is situated in chromosome 9. Majority of the corresponding fusion protein is the p210 major isoform that is transcribed from the b2a2 (e13a2) and b3a2 (e14a2) fusion transcripts. The chimeric BCR::ABL1 p210 oncoprotein is a tyrosine kinase with elevated activity leading to uncontrolled cell division that results in leukemia, particularly in CML cases. With the advent of Tyrosine Kinase Inhibitor (TKI) drugs, CML patient is treated with therapeutic targeting the BCR::ABL1 fusion. Most patients undergoing TKI treatment regime will achieve remission, requiring continual long-term follow-up and disease monitoring. mSEP™ BCR::ABL1 Major dPCR Test serves to support the monitoring of minimal residual disease quantitation of the BCR::ABL1 major isoform by microchamber-based partition digital PCR (dPCR) analysis.

Principle of the Procedure

mSEP™ BCR::ABL1 Major dPCR Test uses target-specific oligonucleotide primers and probes for direct one-step Reverse Transcription (RT) and targets amplification within the microchamber-based partition of digital PCR system (dPCR). BCR::ABL1 translocation b2a2 (e13a2) and/or b3a2 (e14a2) as well as the ABL1 endogenous gene transcripts are simultaneously reverse transcribed, amplified, detected and quantified. Using total RNA extracted from peripheral blood specimen from CML patient diagnosed with p210 major isoform, mSEP™ BCR::ABL1 Major dPCR Test quantitates copies of b2a2 (e13a2) and/or b3a2 (e14a2) fusion transcripts in the FAM channel and ABL1 endogenous control in the HEX channel. mSEP™ BCR::ABL1 Major dPCR Test quantifies the copy of BCR::ABL1 transcript as a ratio of BCR::ABL1 fusion / ABL1 endogenous control copy and returns the calculated value standardized to the International Scale (% I.S.) and log Molecular Reduction (MR) scale.

mSEP™ BCR::ABL1 Major dPCR Test is intended for use on the QuantStudio™ Absolute Q™ Digital PCR System. Reagents in mSEP™ BCR::ABL1 Major dPCR Test are sufficient for 192 test reactions. A set of two % I.S. Calibrator Controls [IS_10% (MR1) and IS_0.1% (MR3)] are included in mSEP™ BCR::ABL1 Major dPCR Test to function as both process and calibration controls that ensure the assay is traceable to the World Health Organization (WHO) First International Genetic Reference Panel (NIBSC Code: 09/138) for the quantitation of BCR-ABL translocation.

There are two measures used for monitoring CML treatment progress:

1. International Scale percent ratio (% I.S.) is calculated by dividing the number of BCR::ABL1 fusion copies by the number of ABL1 endogenous control copies X 100 X Conversion Factor (C.F.) as defined by the % I.S. Calibrator Controls [IS_10% (MR1) and IS_0.1% (MR3)].

2. Log Molecular Reduction (MR) level is a transformed $\log_{10}$ of % I.S.

   Formulae for the reporting format is as follow:

   % I.S. = BCR::ABL1 copies / *ABL1 copies* X 100 X *C.F.*

   $MR = \log_{10}(100\%\ I.S.) - \log_{10}(\%\ I.S.) = 2 - \log_{10}(\%\ I.S.)$

The % I.S. ratio is reported in the linear scale, while the MR is the % I.S. ratio converted to $\log_{10}$ scale and subtracted from the baseline of 100% I.S., which is MR 0.

Kit Content

Catalog Number		MBCR-RA192
Test		**192**
Frozen Component	**-30°C to -10°C**	**Quantity**
mSEP™ dPCR Direct RT MM	Enzyme Mix	1 Tube
mSEP™ BCR::ABL1 Major PPM	Primer Probe Mix	1 Tube
mSEP™ dPCR Nuclease-Free Water	Water	1 Tube
mSEP™ BCR::ABL1 Major IS_10%	Calibrator Control	1 Vial
mSEP™ BCR::ABL1 Major IS_0.1%	Calibrator Control	1 Vial

Calibrator Controls shall be extracted for total RNA in the same way as how blood specimen is extracted for RNA in the laboratory. DNase treatment is recommended during RNA extraction to eliminate residual genomic DNA. To avoid degradation, it is recommended to prepare small aliquot(s) of extracted RNA of IS_10% and IS_0.1%, independently, for storage in -80°C freezer.

Storage Condition

- Store all frozen kit components between -30°C to -10°C upon receipt
- Keep kit components away from light until ready to use

NOTE: **Repeated thawing and freezing may reduce the sensitivity of the assay**

- Keep all frozen kit components on ice block during usage
- Store between -30°C to -10°C after use
- If left unopened, kit components are stable until the expiration date indicated on respective component's label

Materials Required but Not Provided

a) Consumable
- Personal Protective Equipment (PPE)
- Sterile pipette tip with filter
- 1.5 mL microcentrifuge tube
- QuantStudio™ Absolute Q™ MAP16 Plate Kit (Cat. No. A52865)
- Decontamination product

b) Equipment
- QuantStudio™ Absolute Q™ Digital PCR System (Software Release Version: 6.3.2 or higher)
- RNA extraction system
- Microcentrifuge for 1.5 mL microcentrifuge tube
- Micropipette (0.5 to 10 µL, 2 to 20 µL, 10 to 100 µL, 100 to 1000 µL)
- Freezer (-20°C)
- Vortex mixer

c) Additional Accessory
- Ice or cooler unit
- Tube rack / stand

Warnings and Precautions

- All specimens / samples shall be treated as potentially infectious.
- Specimen processing shall be performed in accordance with national biological safety regulations.
- Perform manipulation of blood derived biological specimen within a Class II (or higher) Biological Safety Cabinet (BSC).
- Wear appropriate PPE including, but not limited to, protective disposable glove, laboratory coat and eye protection when handling specimen / sample and kit component. Wash hands thoroughly after handling specimen / sample and kit component.
- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product (DNA amplicon) from previous reactions. Incorrect results could occur if either the sample or kit component used become contaminated by accidental introduction of PCR product (DNA amplicon). Workflow in the laboratory shall proceed in a unidirectional manner.
- Clean and decontaminate work area and instrument, including pipette, with commercially available decontamination product.
- A designated working area shall be dedicated for processing specimen and to add extracted RNA sample to dPCR Mix.
- Recommend using sterile pipette tip with filter.
- Do not use kit component beyond the expiration date shown on respective label.
- Follow laboratory safety rules and procedures as defined by approved biohazard safety guidelines and/or regulations.
- Discard all waste according to local safety regulations.
- RNA sample shall be maintained on ice block or on ice during use to ensure stability.
- Material Safety Data Sheet (MSDS) is available upon request.

Additional Precautions when Handling RNA Samples

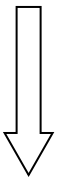
- Designate a separate working area for RNA work only.
- Clean work area and instrument, including micropipette, with 100% ethanol and/or commercially available RNase inactivation reagent.
- Always wear gloves while working with RNA. Avoid touching surface and equipment that are not decontaminated.
- Use only sterile and RNase-free disposable plastic ware.
- Use only nuclease-free water.
- Work quickly on ice block or on ice during use.

Quality Control

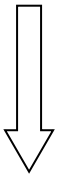
Under Veredus' Quality Assurance (QA) program, the performance of mSEP™ BCR::ABL1 Major dPCR Test is monitored routinely to ensure consistent quality. Sampling is done on every kit lot and Quality Control (QC) tests carried out via amplification of the respective RNA and plasmid template.

Workflow

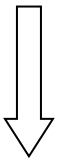
RNA Extraction



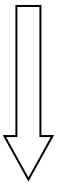
Prepare Reaction Mix



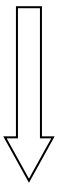
Add Extracted RNA / mSEP™ dPCR Nuclease-Free Water (NTC)



Load 9 µL of Reaction Mix into Reaction Well



Load Plate onto QuantStudio™ Absolute Q™ Digital PCR System and Start Run



Analyze and Report Result

Specimen Collection, Handling and Storage

Specimen shall be collected, handled and stored following the laboratory's standard procedures. Inadequate and/or inappropriate specimen collection, storage and transport are likely to yield false negative results. Training in specimen collection is highly recommended because of the importance of specimen's quality.

Sample Preparation

Total RNA extracted from human peripheral blood is the starting material (sample) for mSEP™ BCR::ABL1 Major dPCR Test. DNase treatment is recommended during RNA extraction to eliminate residual genomic DNA. To obtain maximum performance, it is critical to establish the RNA extraction process. Some naturally occurring substances such as heme and polysaccharides may behave as PCR inhibitor that interferes with the performance of mSEP™ BCR::ABL1 Major dPCR Test. Please refer to the respective manufacturer's handbook for detailed RNA extraction procedure.

The following nucleic acid extraction kits are recommended:

- QIAamp RNA Blood Mini Kit (Cat. No. 52304)
- Maxwell® RSC simplyRNA Blood Kit (Cat. No. AS1380)

All extracted RNA samples shall be quantified and check for purity spectrophotometrically using nanodrop spectrophotometer and diluted preferably to 100 ng/µL (or between 75 to 125 ng/µL) before use.

Setup Procedures

a. Thaw mSEP™ dPCR Direct RT MM and mSEP™ BCR::ABL1 Major PPM on ice block or on ice.
b. Pulse-vortex thawed reagents for approximately 10 seconds then perform a quick spin of mSEP™ dPCR Nuclease-Free Water, mSEP™ dPCR Direct RT MM and mSEP™ BCR::ABL1 Major PPM.
c. Determine the number of reactions to set up. It is recommended to factor excess (5% more) reaction mix for NTC, Calibrator Controls (IS_0.1% and IS_10%) and sample, and for pipetting variations. Refer to table 1.
d. Prepare reaction mix (without adding sample and mSEP™ dPCR Nuclease-Free Water) in microcentrifuge tube according to table below. Mix the reaction mix by pipetting up and down, or pulse-vortex briefly.
e. Quick spin reaction mix for approximately 5 seconds and place microcentrifuge tube on ice block or on ice.

Table 1

No.	Item	Preparation Volume for 1 Reaction (µL)	Preparation Volume for *N* Reactions (µL)
1	mSEP™ dPCR Direct RT MM	2.5	2.5 X 1.05** X *N*
2	mSEP™ BCR::ABL1 Major PPM	1	1 X 1.05** X *N*
3	Sample / Calibrator Controls	0 to 6.5*	As Calculated
4	mSEP™ dPCR Nuclease-Free Water	0 to 6.5*	(0 to 6.5) X 1.05** X *N*
Total		10	10

* Volume should be adjusted accordingly depending on input amount of Sample (400 ng per preparation) or Calibrator Controls (250 ng for preparation)
** It is recommended to factor excess (5% more)

f. Pipette appropriate amount (up to 6.5 µL per preparation) of sample (e.g. "Patient 1") or Calibrator Controls (IS_0.1% and IS_10%) and/or mSEP™ dPCR Nuclease-Free Water into microcentrifuge tube containing reaction mix, accordingly.
[***Note***: *Duplicates for every reaction are required*]
[***Note***: *For NTC, pipette only 6.5 µL of mSEP™ dPCR Nuclease-Free Water to reaction mix*]

g. Pulse-vortex the microcentrifuge tube for approximately 10 seconds.

h. Centrifuge at top speed (approximately 13,000 RPM) for 2 minutes.

i. Set up the plate layout on QuantStudio™ Absolute Q™ Digital PCR System. Sample name for Internal Standards must be *IS_10%* and *IS_0.1%*, respectively, and non-template control as *NTC*.

j. The layout for each run shall have duplicates.
[***Note***: *A total of up to 5 samples can be tested in each QuantStudio™ Absolute Q™ MAP16 plate within a single run. Otherwise, partially used MAP plate can be stored for use on a later date*]

IS_10%	Sample 1	Sample 3	Sample 5
IS_10%	Sample 1	Sample 3	Sample 5
IS_0.1%	Sample 2	Sample 4	*NTC*
IS_0.1%	Sample 2	Sample 4	*NTC*

k. Remove QuantStudio™ Absolute Q™ MAP16 plate from its package. Handle the plate by its frame and remove it from the package only when it is ready to load. Place the plate on a clean, dry surface.

l. Load 9 µL of reaction mix to the bottom of each reaction well in MAP16 plate at an angle of 45 degrees. Pipette to first stop to avoid forming bubbles. Change a new pipette tip for each well.

m. With a new pipette tip, add 15 µL of the isolation buffer to the top side of the well, above the reagent mix. Pipette carefully such that the isolation buffer does not mix with the reaction sits on top of the reaction mix.

n. Carefully fit the gasket over the well by column.
o. Load the plate into QuantStudio™ Absolute Q™ Digital PCR System and start run with the following conditions:

Step	Temperature (°C)	Duration (Sec)	Cycle(s)
RNA-RT	55	600	1x
Preheat	96	600	1x
Denature	96	5	40x
Anneal + Extend	64	10	

p. Fluorescence Detector (Dye)

Target	Detector Dye
BCR::ABL1 Fusion	FAM
ABL1 Endogenous Control	HEX

Passive reference: ROX

q. After run has completed, manually (instead of using “Auto”) set thresholds for both FAM and HEX detection channels accordingly:
- FAM Fluorescence: 5000
- HEX Fluorescence: 2000

r. Export a copy of the raw data as *.csv* and save the run ID as the filename.

Subsequent Procedures (s. to w.) Involve Analyzing Raw Data (*.csv* file) Using mSEP ™ cp Analysis - Optional for Laboratory Use

s. Open the mSEP ™ cp Analysis and log in.
t. Go to Analysis and select the *.csv* file to import for analysis
u. The Run ID, CF Value, and Sample IDs should be automatically detected and pulled for analysis.
v. Select the Assay as *BCR-ABL_Major_v1.0.0* (or higher version) and continue with the analysis.
w. Refer to the Instruction For Use (IFU) of mSEP ™ cp Analysis for detailed instruction.

Assay Controls

The following controls are included:

a. A set of two % I.S. Calibrator Controls [IS_10% (MR1) and IS_0.1% (MR3)] are included in mSEP™ BCR::ABL1 Major dPCR Test to function as both process and calibration controls that ensure the assay is traceable to WHO's first International genetic Reference Panel (NIBSC Code: 09/138) for the quantitation of BCR-ABL translocation.
b. No Template Control (NTC) to monitor contamination risk of kit reagent.

Interpretation of Results

No Template Control (NTC)

NTC reaction shall be negative i.e. no copies detected for both BCR::ABL1 fusion as well as the ABL1 target, in FAM and HEX channels, respectively. If NTC reaction detected significant positive BCR::ABL1 (>1 copy) and/or ABL1 (>10 copies), contamination may have occurred. Invalid the run and repeat with strict adherence to the guidelines.

Calibrator Controls

Both IS_10% (MR1) and IS_0.1% (MR3) consist of cells containing BCR::ABL1 fusion as well as normal cells blended at the expected % I.S. ratio.

Make sure that NTC and reported % I.S. values are as expected:

NTC
Range: 0 ≤ no. of BCR::ABL1 copy ≤ 1
Range: 0 ≤ no. of ABL1 copy ≤ 10

IS_0.1%
Range: 0.0360 ≤ % I.S. value ≤ 0.4553

IS_10%
Range: 3.6127 ≤ % I.S. value ≤ 37.4603

If not, invalid the run. Determine the cause of failure and implement appropriate corrective action(s).

Limitations of the Test

- Strict compliance with this IFU is required for optimal results. Modification(s) to these procedures may alter performance of this test.
- Appropriate specimen collection, handling, storage and processing procedures are required for optimal performance of this test.
- This test is not intended to be used on specimen directly. Specimen needs to be processed using appropriate RNA extraction method prior to using this test.
- Results from this test can be interpreted with other laboratory data and clinical information, whenever necessary.
- Use of this kit shall be limited only to trained laboratory professionals who are proficient in performing RT-dPCR assay on the QuantStudio™ Absolute Q™ Digital PCR System.
- This test is designed for high specificity and sensitivity, but a low incidence of false results may occur. A negative result does not preclude the possibility of existence of the target mutation.
- This test provides a quantitative value for the detected target BCR::ABL1 fusion as well as the ABL1 copy.
- This test does not differentiate between b2a2 (e13a2) and b3a2 (e14a2) fusion transcript, and does not monitor other rare fusion transcripts resulting from t(9;22).
- This test is not intended for the diagnosis of CML and other atypical fusion transcripts.
- Mutation(s) within gene target regions covered by primers and/or probes used in this test may result in failure to detect target fusions.
- False negative results may occur due to, but not limited to, presence of sequence variants in the fusion breakpoint target(s) of the assay, procedural error(s), amplification inhibitor(s), or inadequate nuclei acid for amplification.
- False positive results may occur due to, but not limited to, genomic DNA contamination or non-specific signal(s) in this test. DNase treatment is recommended as part of the RNA extraction process to eliminate as much residual genomic DNA as possible.

Performance Characteristics

1. Analytical Sensitivity (Limit of Detection LoD)

   The LoD of the test was determined by using characterized stock of total RNA extracted from human cell line standards traceable to WHO's first International genetic Reference Panel (NIBSC Code: 09/138) for the quantitation of BCR-ABL translocation. The panel of five levels of % I.S. standard offers a dynamic range from 10% to 0.0032%, allowing analytically validation extended to reportable range down to the lowest level of Molecular Response (MR) 4.5 as required by NCCN. The LoD was defined as the lowest detectable % I.S. or MR level at which 95% of all replicates are tested positive at this level.

2. Analytical Specificity

   Analytical specificity supports detection of BCR::ABL1 fusion transcripts [b2a2 (e13a2) and b3a2 (e14a2)] exclusively, and no cross reactivity with BCR::ABL1 fusion transcript e1a2 (p190) to at least MR 4.5 level.

3. Repeatability and Reproducibility

   The repeatability and reproducibility of this test were validated by performing three rounds of testing on three different days by three different operators. Each round of testing included 20 replicates from three kit lots. Total RNA extracted from % I.S. human cell line standards was used as sample. The variation of calculated % I.S. and MR values detected within each round represents the repeatability of this test and the overall variation of % I.S. and MR values across three rounds represents the reproducibility of this test.

Disposal

Dispose of hazardous or biologically contaminated materials according to local safety regulations.

Technical Assistance

If you have any questions or technical issues regarding the use of this kit, or any other Veredus' products, please contact our technical support.

Contact

Your opinion(s), comment(s), question(s) or feedback are important to us and all Veredus' customers. Please contact us if you have any suggestions about product's performance or new application(s) and technique(s).

For information and technical assistance, please contact us via:

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Understanding the Symbols

Symbol	Meaning
	Catalog number
	Lot number
	Contains sufficient for <n> tests
	Manufacturer
	Temperature limitation
	Use-by date (YYYY-MM-DD)
	Consult Instructions for Use

Product Use Limitations, Warranty Disclaimer

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