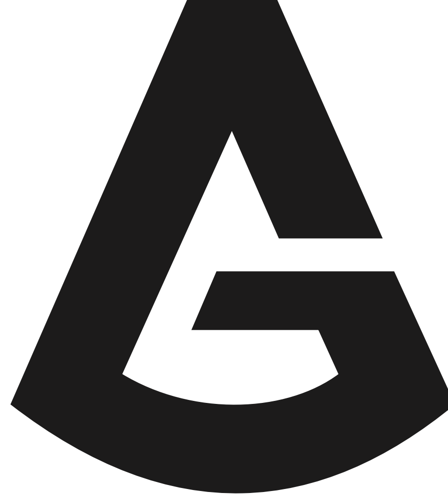

556875|MLL-AF4 *t(4;11) (q21;q23)*

25 rxn

aRTegen™ MLL-AF4 quantitative RT-qPCR kit Instructions for Use

Artegen Sağlık ve Teknoloji Limited Şirketi

Trakya Teknopark Edirne Türkiye, 22030

E-mail: info@artegenomics.com

support@artegenomics.com

Intended Use

The **aRTegen^{TM} MLL-AF4 quantitative qPCR kit** is intended for quantitation of MLL-AF4 (t(4;11) (q21;q23)) fusion gene transcripts in peripheral blood or bone marrow samples.

General Information

Mixed Lineage Leukemia (*MLL*) gene arrangements are most frequent genetic alterations among human leukemia. Translocation occurs by the in-frame fusion of the amino-terminal end of MLL with one of more than 50 proteins. Among them, MLL–AF4 acute lymphocytic leukemia (ALL) results from a balanced translocation between MLL and AF4 (t(4;11)(q21;q23)) with the frequency of 5–6% in infant, child and adult ALL cases and it has a poor prognosis. Although the role of MLL gene in mammalian cells is not well understood, it is thought that MLL has a key role in segmentation during development. At the molecular level, translocation breakpoints in MLL and AF4 genes are distributed within introns, between exon 8 and exon 12(MLL) and exon 3 and exon 7(AF4). Some transcripts are more frequent in either adult or infant ALL and produce different MLL-AF4 transcription product in different size. Detection and quantification of these MLL-AF4 fusion transcripts in pediatric ALL is crucial for determination of prognosis and therapeutic approaches. The **aRTegen^{TM} MLL-AF4 quantitative qPCR kit** can be used for detection and quantification of MLL-AF4 fusion transcripts for monitoring transcript levels.

Product Description

The **aRTegen^{TM} MLL-AF4 quantitative qPCR kit** detect and quantify **MLL-AF4** fusion gene transcripts based on reverse transcription and real time polymerase chain reaction (qPCR) principle by using specific primer sets and fluorescence labeled hydrolysis (TaqMan) probes. The kit includes reagents and enzymes to detect and amplify targets specifically in two different fluorescence channels. FAM labeled hydrolysis probe detects **MLL-AF4** fusion transcripts as a target and HEX labeled hydrolysis probe detect ABL1 gene transcript as an internal control. Two step qPCR reaction enable the test to be highly sensitive and to work well with limited sample amount. Separate optimization of Reverse transcription and qPCR steps and ability to generate cDNA stock for further use also makes the kit more advantages over commercial kits.

Kit Content

This kit includes the materials listed in table below. The kit content is prepared for performing 25 reactions sufficiently.

Item (25 rxn)	Quantity	Volume / Reaction
MLL-AF4 primer probe Mix	1 x 95 µL	2,75 µL
ABL primer probe Mix	1 x 70 µL	2 µL
2x qPCR Master Mix	1x 840 uL	12.5 µL
Non template Control	1x 500 µL	2.5 µL
MLL-AF4 standard 1 (100 copies/µl)	1x 15 µL	2.5 µL
MLL-AF4 standard 2 (1000 copies/µl)	1x 15 µL	2.5 µL
MLL-AF4 standard 3 (100000 copies/µl)	1x 15 µL	2.5 µL
MLL-AF4 standard 4 (1000000 copies/µl)	1x 15 µL	2.5 µL
ABL standard 1 (10 copies/µl)	1x 15 µL	2.5 µL
ABL standard 2 (100 copies/µl)	1x 15 µL	2.5 µL
ABL standard 3 (1000 copies/µl)	1x 15 µL	2.5 µL
ABL standard 4 (10000 copies/µl)	1x 15 µL	2.5 µL

Sample Collection, Handling and Transportation

Collecting human peripheral whole blood sample in EDTA vial is strongly recommended. To use 2 to 5 ml blood sample is recommended. Purified RNA sample is the starting material for the reverse transcription and further qPCR assay. Transportation and storage of specimens is recommended at 4-8°C and handling of specimens is recommended within 48 hours upon collection.

Important Note: Avoid from freezing because of hemolysis risk of blood samples. Please also note that the kit does not supply equipment or reagents for sample collection and RNA extraction.

Storage & Transportation of the Kit

The kit is transported on gel ice. All components should be stored in -20°C upon arrival of the kit. For the expiration date please refer to the label on the box. Please avoid repeated freeze-thaw cycles as this might affect the performance of the test. Keeping reagents at 2 to 8°C is not suggested for a longer time than 4 hours. If a study is planned as intermittent, please freeze it in aliquots.

Materials and equipment required by the user

- ipsogen RT Kit (Qiagen)
- Real-Time PCR Instrument with FAM and HEX channels
- RNA extraction system or column-based purification kit for extraction of nucleic acid samples.
- Micropipette (Variable)
- Micropipette tips (DNase and RNase free)
- Quick Spin Centrifuge: min. 3000 rpm
- 1.5- or 2-mL microcentrifuge tubes
- qPCR tube/strip and cap or qPCR Plate and seal: Compatible with Real-Time PCR Instrument, nuclease-free.
- UV Cabinet for PCR Setup
- Cold Tube Rack (for microcentrifuge tubes and PCR tubes/strips)
- Disposable powder-free nitrile gloves
- Please check instruments for calibration, proper installation and maintenance as in recommended by manufacturers.

RNA extraction

Total RNA is extracted from whole blood by using spin column based nucleic acid purification kits or common sample preparation methodologies compatible with RT-qPCR principle. The quality of the purified RNA sample affects the performance of the entire assay. The recommended RNA concentration should be >50 ng/µl and the RNA purity (OD_{260}/ OD_{280} ratio) should be > 1.7.

Reverse Transcription

Reverse Transcription step is performed by following ipsogen RT Kit (Qiagen) procedure provided by the kit.

Real Time PCR (qPCR)

Before to start:

- All reagents should be kept on a cold block at 2-8 °C.
- Thaw all reagents before use. Vortex and briefly spin down Standards and Prime-Probe mixes. DO NOT vortex the master mix. Invert thoroughly to mix and centrifuge briefly before to use.
- Pay attention extremely to prevent cross-contamination.

Preparation of RQ-PCR Mix

1. Prepare the qPCR master mix as in table1 and table2 below. For ''n'' sample, use ''n+10'' because of 8 for standards and 2 for negative control reaction. For each sample also use 2 tubes, 1 tube for ABL master mix and 1 tube for **MLL-AF4** master mix separately.

Components	Volume Per Reaction	Instructions
2x QuantiTect Probe PCR Master Mix	12.5 µl	Thaw and mix by inverting, spin down (DO NOT vortex)
ABL primer probe mix	2 µl	Thaw, vortex thoroughly, spin down.
RNase-free ddH2O	8 µl	
Purified Sample RNA (ABL standards or nuclease free water (NTC))	2.5 µl	Pipetting (DO NOT) vortex.
Total reaction volume	25 µl	Make sure there is no solution remaining in the cap by making spin down before loading into PCR machine.

Table 1: Sample tube for ABL master mix

Components	Volume Per reaction	Instructions
2x QuantiTect Probe PCR Master Mix	12.5 µl	Thaw and mix by inverting, spin down (DO NOT vortex)
MLL-AF4 primer probe mix	2,75 µl	Thaw, vortex thoroughly, spin down.
RNase-free ddH2O	7,25 µl	
Purified sample RNA (MLL-AF4 standards or nuclease free water (NTC))	2.5 µl	Pipetting (DO NOT) vortex.
Total reaction volume	25 µl	Make sure there is no solution remaining in the cap by making spin down before loading into PCR machine.

*Table 2: Sample tube for **MLL-AF4** master mix*

1. Invert the tube at least 5 times for proper mixing and centrifuge briefly.
2. Add 22.5 µl of qPCR master mix into PCR tubes.
3. Add 2.5 µl of each sample's nucleic acids (or standards) into the PCR tubes containing the qPCR master mix.
4. Close each tube gently and eliminate all air bubbles. Spin down the tubes in a microcentrifuge.
5. Place the reaction tubes in the real time thermal cycler and set the cycling parameters as in the table below.

Setting for qPCR			
Stage	**Temp**	**Time**	**Cycling**
Initial incubation	95 °C	15 min	1
Initial denaturation	94°C	15 sec	**50** cycles
Annealing	60 °C	1 min	

**** The kit is optimized in Rotorgene Q instruments, different instruments may display variation in Ct values and they require further optimization at their discretion.**

Setting for Thermal Cycler

Reaction Volume	25µl
Fluorescence Dyes	FAM & HEX
Passive reference None	None

Settings for Real Time PCR Instruments

Data Acquisition	**Target**
FAM	**MLL-AF4**
HEX	**ABL** (control)

Shelf life of the kit is 18 months; check the box for the expiration date. Each reagent can be used until the expiration date printed on the tube. The kit's expiration date is determined by the expiration date of the reagents.

Analysis and Reporting

qPCR data analysis should be performed by personals who have experience with qPCR technique and data interpretation. Please check the recommendations of manufacturers for the relevant instruments.

Note: For the Qiagen Rotor-Gene Q system, the analysis should be performed by setting the threshold for each channel at 0.1. Different instruments may display variation, and they require further adjustment of the thresholds at their discretion.

Test Tubes	**Interpretation of NTC and Standards**		
	MLL-AF4 (FAM)	**ABL** (HEX)	Results Interpretation
Non-Template Control (NTC)	No amplification	No amplification	VALID; (no contamination)
	Amplification	Amplification	INVALID; contamination of samples or environment, eliminate the contamination by using new set of reagents and provide decontamination of work space, etc.
	Amplification	No amplification	
	No amplification	Amplification	
Standards	There should be 10% more or less differences between calculated copies/µl and the expected copies/µl of each standard		VALID; (It should be three Ct differences between standards)
	No amplification	No amplification	INVALID; The assay must be repeated.

Test Tubes	**Interpretation of Patient Results**		
	MLL-AF4 (FAM)	**ABL** (HEX)	Results Interpretation
Patient Sample	No amplification	Amplification $21.9 \leq Ct \leq 29.3$ ($1.3x10^3$-$1.3x10^5$ copies)	Negative
	Amplification $20 \leq Ct \leq 40$	Amplification $21.9 \leq Ct \leq 29.3$ ($1.3x10^3$-$1.3x10^5$ copies)	Positive, calculate the ratio

Calculations:

Calculations is made by considering normalized copy number (NCN) of fusion gene. Perform calculations by using the excel sheet provided by the kit.

MLL-AF4 (%) = [(Copies/µl of MLL-AF4)/Copies/µl of ABL)]x100

Analytical Specifications

- **Limit of Detection (LoD):**

To calculate minimum copy number of **MLL-AF4** transcript that can be detect by the assay, serial dilution of **MLL-AF4** positive patient sample cDNA was prepared and run with the standard and control sample. From the standard curve calculations, limit of detection of the assay was found less than 100 copies per **MLL-AF4** and ABL transcripts.

Limitations

1. Under the conditions described below, the results may be false negative & positive;

1.1. If the concentration of the specimen is low or improperly collected, the result may be false negative.

1.2. If processing & transportation is not suitable for the given conditions, the result may be false negative.

1.3. Inappropriate storage of the specimen may lead to false negative result.

1.4. In the presence of the PCR inhibitors, the result may be false negative.

1.5. Occurring cross-contamination or non-specific bindings during sample processing may cause false positive results.

Cross Reactivity

The assay does not show any cross reactivity with known human transcripts, pathogens and not interfere with human genomic DNA. Laboratory studies demonstrate that the kit is highly specific to **MLL-AF4** and ABL transcripts.

References

1. Gabert, J., Beillard, E., van der Velden, V. H., Bi, W., Grimwade, D., Pallisgaard, N., Barbany, G., Cazzaniga, G., Cayuela, J. M., Cavé, H., Pane, F., Aerts, J. L., De Micheli, D., Thirion, X., Pradel, V., González, M., Viehmann, S., Malec, M., Saglio, G., & van Dongen, J. J. (2003). Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia - a Europe Against Cancer program. Leukemia, 17(12), 2318–2357. https://doi.org/10.1038/sj.leu.2403135