

Classification of Leukemia



Leukemia, commonly known as **blood cancer**, is a malignant disease of the blood system.

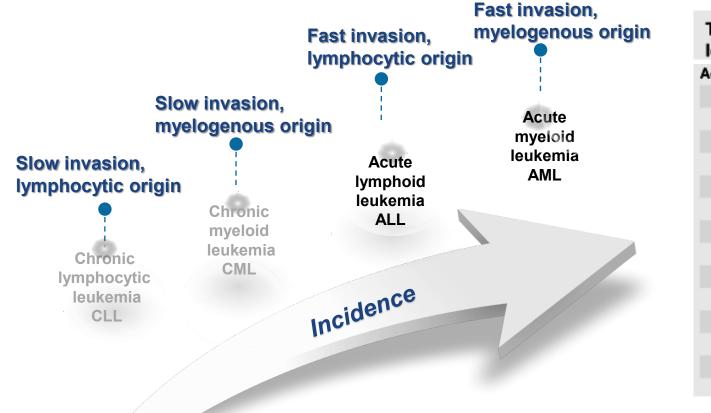


Table 1. WHO classification of myeloid neoplasms and acute leukemia
Acute myeloid leukemia (AML) and related neoplasms
AML with recurrent genetic abnormalities
AML with t(8;21)(q22;q22.1);RUNX1-RUNX1T1
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);CBFB-MYH11
APL with PML-RARA
AML with t(9;11)(p21.3;q23.3);MLLT3-KMT2A
AML with t(6;9)(p23;q34.1);DEK-NUP214
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2, MECOM
AML (megakaryoblastic) with t(1;22)(p13.3;q13.3);RBM15-MKL1
Provisional entity: AML with BCR-ABL1
AML with mutated NPM1
AML with biallelic mutations of CEBPA
Provisional entity: AML with mutated RUNX1
AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms

The fusion genes are used for standard

leukemia classification.

Clinical Significance of Fusion Genes Detection

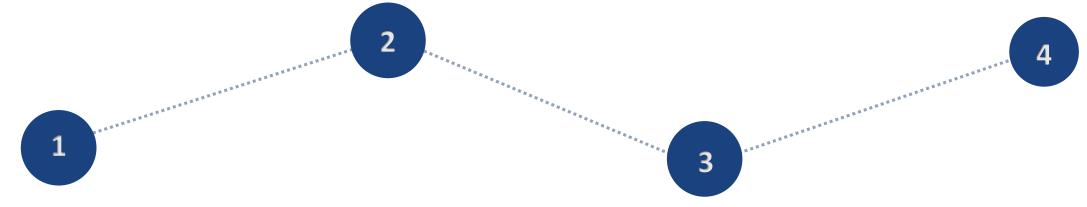


Disease Prognosis

AML1-ETO: Commonly seen in AML-M2 patients, and a factor for better prognosis **MLL-AF4**: poor prognosis, patients need to be given a high-risk treatment

Minor Residual Disease Monitoring

When treatment reaches the early stage of complete remission, MRD is tested periodically and the treatment method, regimen and duration of treatment are adjusted according to the level of MRD



Drug Prediction

BCR-ABL1: predicting that patients can benefit from the targeted drug Gleevec

PML-RARa: predicting that retinoic acid and arsenic trioxide therapy is effective on patients

Treatment Process Monitoring

Indicate the changes of tumor cells in the course of treatment by measuring the fusion gene content to monitor the effect of treatment

Full Solution to Leukemia Fusion Genes Detection

Initial Diagnosis Fusion Genes Screening (LENA Q51) Genotyping Kits (BCR-ABL1, PML-RARα) ALL Diagnosed ; BCR-ABL1 Negative

Ph-like Screening (30 fusion genes & an independent prognosis factor) Ph-like Genotyping (Ph ABL1, Ph ABL2, Ph JAK2, Ph PDGFRB)

Poor effects with TKI therapy

Treatment Process

Single gene quantification (53 fusion genes: Q51 & WTI)

ABL kinase mutation screening

MRD Monitoring

Single gene quantification (53 fusion genes: Q51 & WTI)



Open Platform for Mainstream Fluorescent PCR Instruments



Applicable for instruments with FAM, HEX/JOE, ROX and Cy5 detection channels.*



* The list is non-exhaustive

LIST OF TESTS











LENA PML-RARA V



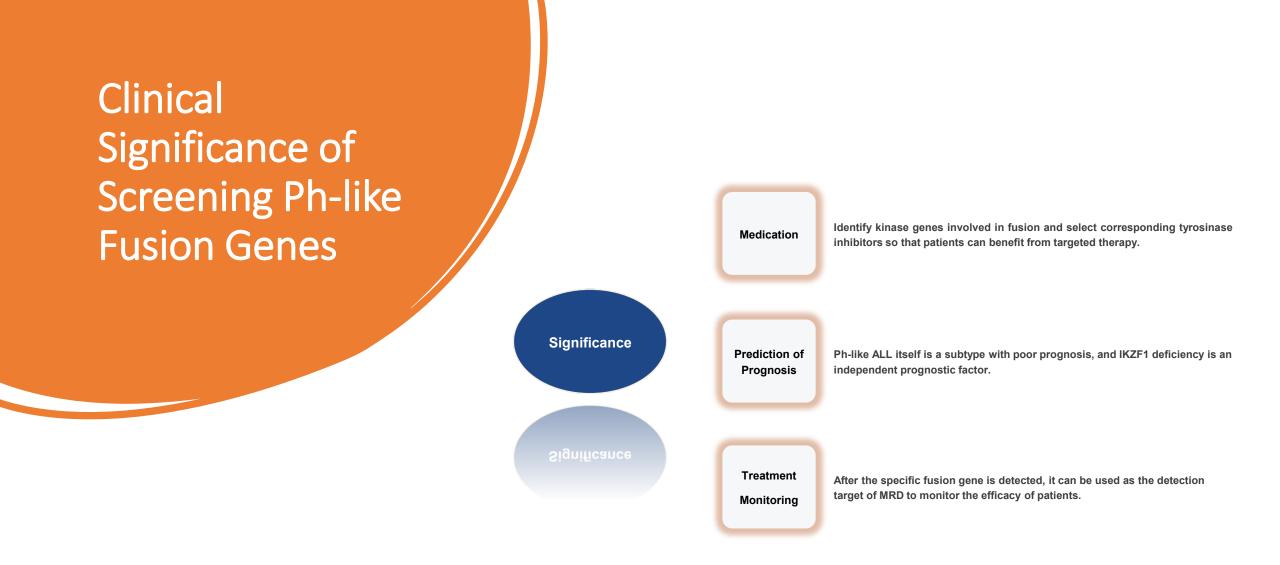
LENA Q51 Leukemia Fusion Genes Screening Kits



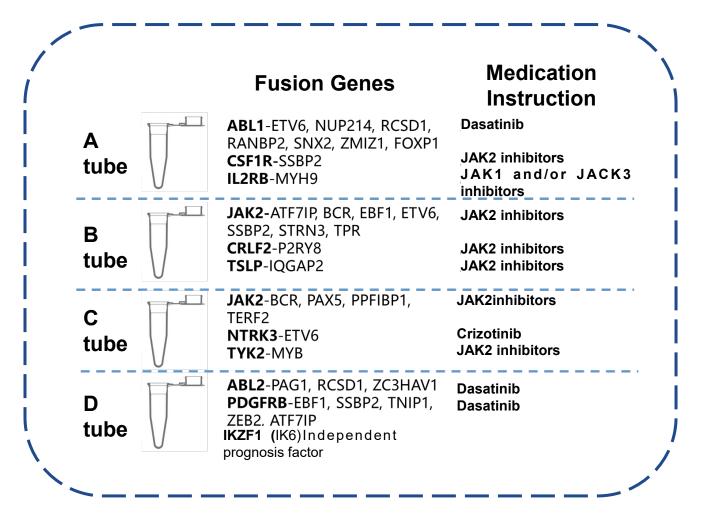


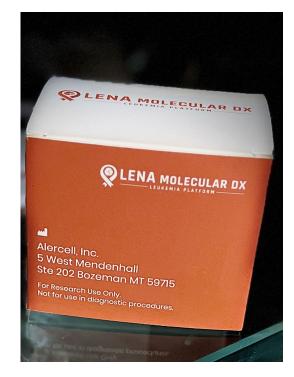
Easier, Faster, and More Comprehensive

	LENA Q51	HemaVision 28Q
Tests/Kit	20	12
Fusion genes	52	28
Breakpoints	200	145
Reverse Transcription Time	15 mins	≥60 mins
System Configuration Time	20 mins per sample	40 mins per sample
Run Time	2h45m	4h
Sample Type	blood or bone marrow	blood or bone marrow
Number of PCR Tubes per Test	12	23
Storage Temperature	-25°C ~ -18°C	-20°C
Postive Control	contained in the kit	additional purchase required
Automatic Extraction System	Yes	No



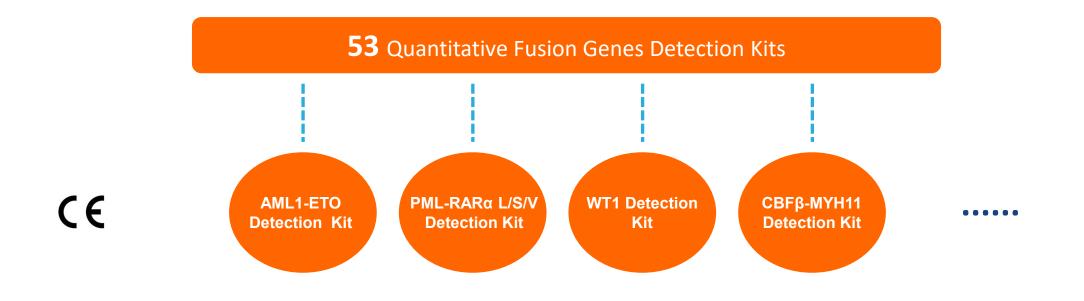
LENA's Ph-like Screening Solution





- Four-tube; four-color reaction system
- Pre-loaded lypholized reagent: no solution dispensing required, making it easier for transportation and storage
- Detects 51 fusion genes and one independent prognostic factor

Comprehensive Quantitative Detection Kits



The most comprehensive fusion genes quantitative detection reagent to satisfy various clinical needs.

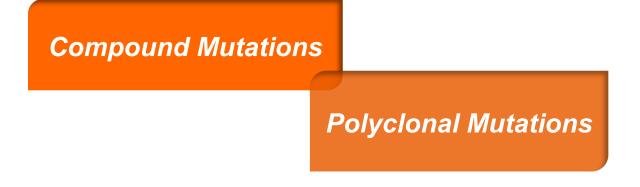
Importance of Detecting BCR-ABL1 KD Mutations

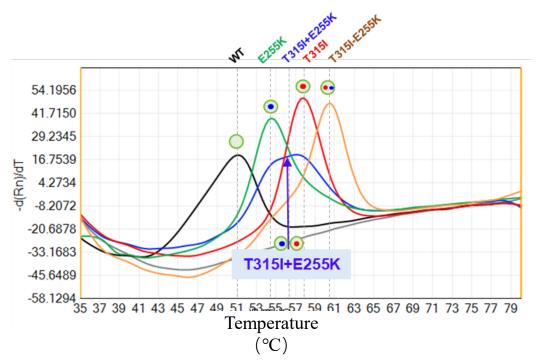
BCR-ABL1 kinase domain (KD) mutations are the most common known cause of resistance to tyrosine kinase inhibitors (TKIs) in CML. Mutation analysis is critical for selection of subsequent TKI therapy after treatment failure. In recent years, more and more studies have shown that compound mutations have stronger TKI resistance.

TKI Drugs		Mutation Sites
Resistance to first-generation drugs	Imatinib	M244V, L248R, V299L, G250E, Y253H, Y253F, E255K, E255V, D276G, T315I, F317L, F317V, F317I, F317C, M351T, E355G, F359C, F359V, F359I, L384M, L387M, V379I, H396R, H396P, S417Y, E459K, F486S
Resistance to second-generation drugs	Nilotinib	Y253H, Y253F, E255K, E255V, F359C, F359V, F359I, T315I
	Dasatinib	V299L, T315A, F317L, F317V, F317I, F317C, T315I
	Bosutinib	E255K, T315I, V299L

Innovative Solution to BCR-ABL1 KD Mutations Detection

- 40 detection sites and over 91% of ABL kinase mutations covered
- Up to 5% Sensitivity, reliable than Sanger
- Easy Operation and interpretation with four tubes and four-color reaction system.
- Results in 5.5 hours: faster and cheaper than NGS





The first rapid identification of compound mutations and polyclonal mutations



BCR-ABL1 refers to a gene sequence found in an abnormal chromosome 22 of some people with certain forms of leukemia.

Unlike most cancers, the cause of chronic myelogenous leukemia (CML) and some other leukemias can be traced to a single, specific genetic abnormality in one chromosome.

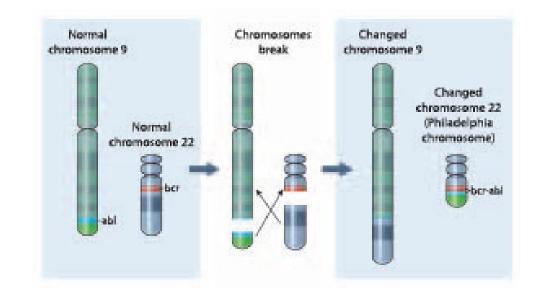
The presence of the gene sequence known as *BCR-ABL1* confirms the diagnosis of CML and a form of acute lymphoblastic lymphoma (ALL), specifically a type of B-lymphoblastic leukemia/lymphoma.

In very rare cases, the abnormal chromosome is linked to cases of acute myeloid leukemia and T-lymphoblastic leukemia/lymphoma.



• The resulting Philadelphia chromosome contains an abnormal *BCR-ABL1* fusion gene that encodes an abnormal protein that is responsible for the development of CML and a type of ALL. At diagnosis, 90-95% of cases of CML show a characteristic t(9;22) *BCR-ABL1* reciprocal chromosomal translocation. About 30% of adults with B-ALL have the translocation, while it is only present in about 2 to 4% of cases in children.

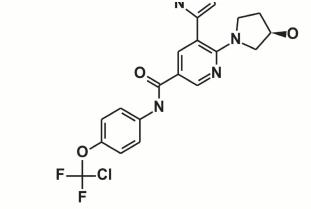
• The protein coded for by the abnormal *BCR-ABL1* fusion gene is a type of enzyme called a tyrosine kinase. That enzyme is responsible for the uncontrolled growth of leukemic cells. When large numbers of abnormal leukemic cells start to crowd out the normal blood cell precursors in the bone marrow, signs and symptoms of leukemia start to emerge. Treatment of these leukemias typically involves a tyrosine kinase inhibitor (TKI).





• Testing for *BCR-ABL1* detects the Philadelphia chromosome and *BCR-ABL1* fusion gene or its transcripts, which are the RNA copies made by the cell from the abnormal stretches of DNA. The presence of the *BCR-ABL1* abnormality confirms the clinical diagnosis of CML, a type of ALL, and rarely acute myeloid leukemia (AML). Treatment for myeloid leukemia (A kinase inhibitor)





LENA BCR-ABL1 kinase domain mutation detection adopts multi-color melting curve technology to monitor drug resistance in patients in time

- 4-tube 4-color reaction system, easy to operate and easy to interpre
- 40 mutation sites, covering more than 91% of ABL kinase mutations
 - The sensitivity is up to 5%, which is higher than Sanger sequencing and the detection result is reliable
 - The operation process same as qPCR, and the result can be obtained in 5.5 hours. Faster & cheaper vs. NGS

Coveri	Covering More than 91% of ABL kinase mutation sites, enabling more accurate, easier and more convenient TKI resistance						
Nr.	MUTATIONS SITES	Nr.	MUTATION SITES	Nr.	MUTATION SITES	Nr.	MUTATION SITES
1	p.M244V(c.730A ≥ G)	11	p.V3791 (c.1135G ≥A)	21	p.E225V (c.764A ≥T)	31	p.V299L (c.895G ≥T)
2	p.L248R (c.743T ≥G)	12	p.S417Y (c.1250C ≥A)	22	p.T315I (c.944C≥T)	32	p.L248V (c.742C ≥G)
3	p.G250E (c.749G ≥A)	13	p.E459K (c.1375G ≥A)	23	p.F317L (c.951C ≥G)	33	p.252H (c.756G ≥C)
4	p.D276G (c.827A ≥G)	14	p.F486S (c.1457T ≥C)	24	p.F317L (c.951C ≥A)	34	p.252H (c.756G ≥T)
5	p.M351T (c.1052T ≥C)	15	p.Y253H (c.757T ≥C)	25	p.F317L (c.949T ≥C)	35	p.L298V (c.892CG)
6	p.E355G (c.1064A ≥G)	16	p.Y253F (c.758A ≥T)	26	p.F317V (c.949T ≥G)	36	p.F311I (c.931T≥A)
7	p.L384M (c1150C ≥ A)	17	p.E255K (c.763G ≥A)	27	p.F317I (c.949T ≥A)	37	p.Y353H (c.1057T ≥C)
8	p.L387M (c.1159T ≥A)	18	p.F359C (c.1076T ≥G)	28	p.F317C (c.950T ≥G)	38	p.E355A (c.1046A ≥C)
9	p.H396R (c.1187A ≥G)	19	p.F359V (c.1075T≥G)	29	p.T315A (c.943A ≥G)	39	p.E450G (c.1349A ≥G)
10	p.H396P (c.1187A ≥C)	20	p.F359I (c.1075T≥A)	30	p.V299L (c.895G ≥C)	40	p.E459G (c.1376A ≥G)

LENA BCR-ABL1 Kinase Domain Mutation Screening Kit

- dually labeled probes to target amplicons generated by PCR;
- then uses melting curve analysis to generate the minus derivative of the fluorescence intensity versus temperature, and
- compares the melting temperature (Tm) to gain mutation information of the sequences.
- LENA BCR-ABL1 is suitable for realtime PCR cyclers with FAM, HEX, ROX and Cy5 detection channels with melting curve analysis function.



The kit can only detect 40 resistance mutations from exon 4 to exon 9 of ABL1 gene, other BCR-ABL1 kinase domain mutations are not covered.

BCR ABL1 Kinase region mutation detection effectively drug use

TKI DRUGS		MUTATION SITES
Resistance to First Generation druges	Imaninib	M0244V, L248R, V299L, G250E, Y253H, Y253F, E255K, E255V, D276G T315L, F317L, F317V, F317C, M351T, E355G, F359C, F359V, F359L L384M, L387M, V379L, H396R, H396P, S417Y, E459K, F486S
	Nitotinib	Y253H, Y253F, E255K, E255V, F359C, F359V, F359I, T315I
Resistance to second Generation Drugs	Dasatinib	V299L, T315A, F317L, F317V, F317I, F317C, T315I
	Bosutinib	E255K, T315I, V299L



TEST PROCEDURE

- 1. Sample collection: Blood or bone marrow
- 2. Extraction of Total RNA
- 3. Reverse Transcription
- 4. Preamplification reaction
- 5. PCR and melting curve analysis.
- 6. Interpretation of the results

LENA – BCR ABL1 – Interpretation of results (I)



Detection Channel	Channel Type of Mutation		
	Nucleic Acid Variation	Protein Variation	
	C.1135G ≥ A	V3791	62.15=1.0C
A-FAM	C.1076T≥G	F359C	70.20=1.0C
	c.944C≥T	T3151	82.39=1.0C
A-HEX			70.22=1.0C
	c.730A≥G	M244V	57.40=1.0c
	c.1187A≥G	H396R	61.68=1.0C
A-ROX	c.827A≥G	D276G	65.86=1.0C
	c.1052T≥C	M351T	70.01=1.0C
	c.749G≥A	G250E	64.78=1.0C
ACy5	c.1159T≥A	L387M	70.22=1.0C
	c.951C≥G	F317L	80.11=1.0C
	c.1187A≥C	H396P	68.28=1.0C
B-FAM	c.1150C≥A	L384M	82.29=1.0C
B-HEX			65.41=1.0C
	c.892C≥G	L298V	57.18=1.0c
	c.1064A≥C	E355A	61.78=1.0c
B-ROX	c.931T≥A	F311I	67.46=1.0c
	c.1064A≥G	E355G	71.70=1.0c
	c.756G≥C	Q252H	85.66=1.0c
	c.743T≥G	L248R	64.59=1.0c
B-Cy5	c.756G≥T	Q252H	69.12=1.0c
	Cc.895G≥C	V299L	64.03=1.0c
C-FAM	c.949T≥A	F317I	69.53=1.0c
	c.758A≥T	Y253F	77.91=1.0c
	c.1349A≥G	E450G	82.34=1.0c





BCR-ABL1 Genotyping Kit is a qualitative test for differentiating of BCR-ABL1 p210, BCR-ABL1 p190, BCR-ABL1 p230 three splice variants with leukemia. The kit detects RNA transcripts of fusion genes extracted from human bone marrow or whole blood samples with BCR-ABL1.THE results obtained from the kit provides more information of BCR-ABL1 to professionals for comprehensive consideration associated with patient condition, drug indication, therapeutic response and other detection findings.

PRINCIPLE

BCR-ABL1 Genotyping Kit is a RT-qPCR based assay for differentiating the subtype of BCR-ABL1 p210, BCR-ABL1 p190, BCR-ABL1 p230. Included in the kit are RT reaction mix and qPCR mix. cDNA is synthesized by adding purified total RNA to the RT reaction mix. The resulting cDNA is added to qPCR reaction tube, which contain specific PCR primers and probes for detection of fusion genes and an internal control gene of GUSB. The qPCR is performed in a real-time thermal cycler with optical filters for the detection of FAM, HEX, ROX and Cy5 fluorescence signals. Amplification plots and the resulting Cq (Quantification cycle) values are used for the identification of the fusion gene subtype. Detection of the GUSB gene is an internal control for the integrity of the RNA sample and functionality of both cDNA and qPCR reactions.





• BCR-ABL1 p210 Kit is intended to quantify the BCR-ABL1 p210 transcripts in human bone marrow or whole blood samples of leukemia patients. The results obtained from the kit provides information about efficiency of treatment or Minimal Residual Disease (MRD) in patients.

• Principle

• BCR-ABL1 p210 Kit is a RT-qPCR based assay for quantification of BCR-ABL1 p210 transcripts. The kit contains RT reaction mix and qPCR mix. cDNA is synthesized by adding purified total RNA to the RT reaction mix. The resulting cDNA is added to 2 PCR reaction tubes, which contain specific PCR primers and probes for BCR-ABL1 p210 and ABL1. The qPCR is performed in a real-time thermal cycler with optical filters for FAM dye. After PCR, a linear regression curve is calculated from the standards for each gene and used for calculating the copy number of each gene in an unknown sample.

BCR-ABL1 p210 transcripts levels will be expressed as percentage of BCR-ABL1 p210 over ABL1.





BCR-ABL1 p190 Kit is intended to quantify the BCR-ABL1 p190 transcripts in human bone marrow or whole blood samples of leukemia patients. The results obtained from the kit provides information about efficiency of treatment or Minimal Residual Disease (MRD) in patients.

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• Principle

• BCR-ABL1 p190 Kit is a RT-qPCR based assay for quantification of BCR-ABL1 p190 transcripts. The kit contains RT reaction mix and qPCR mix. cDNA is synthesized by adding purified total RNA to the RT reaction mix. The resulting cDNA is added to 2 PCR reaction tubes, which contain specific PCR primers and probes for BCR-ABL1 p190 and ABL1. The qPCR is performed in a real-time thermal cycler with optical filters for FAM dye. After PCR, a linear regression curve is calculated from the standards for each gene and used for calculating the copy number of each gene in an unknown sample. BCR-ABL1 p190 transcripts levels will be expressed as percentage of BCR-ABL1 p190 over ABL1.



LENA Pan MPN qPCR Panel kit

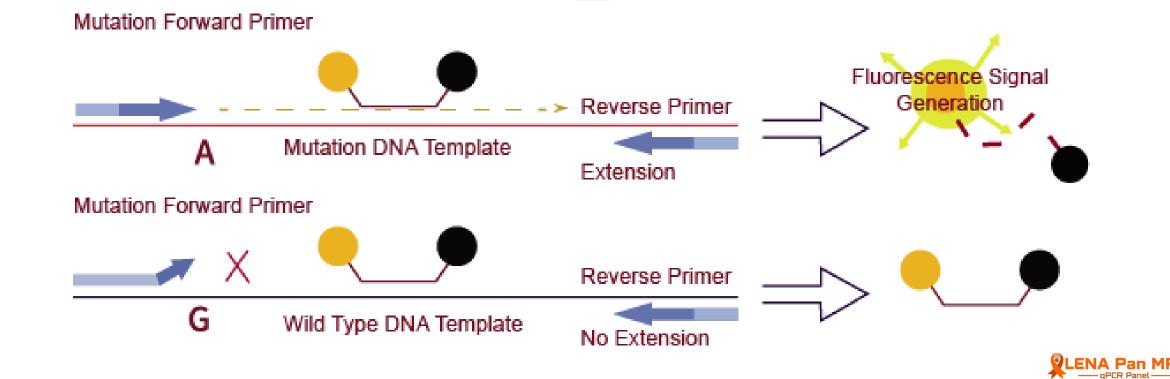
• The MPN-associated Gene Mutation Detection Kit is an in vitro nucleic acid amplification test for the qualitative detection of JAK2, CALR and MPL gene mutations in genomic DNA from blood sample.

• Results are representing the test results of CALR TYPE1/TYPE2, MPL W515/S505N, JAK2 V617F/K539L/exon 12 del, which can be used for clinical diagnosis of Myeloproliferative Neoplasms (MPN).

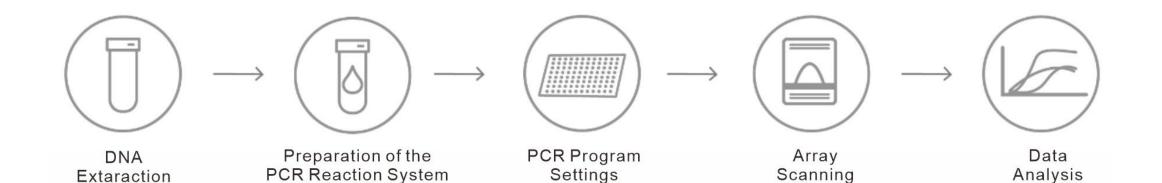


PRODUCT PRINCIPLE

• Myeloproliferative neoplasms (MPN), previously called myeloproliferative disorders (MPD) are a group of diseases that are caused by an overproduction of one or more blood cell types (red cells, white cells or platelets) in the bone marrow. Myeloproliferative neoplasms (MPNs) are associated with dysregulation of tyrosine kinases, leading to abnormal downstream signaling pathways and increased cellular proliferation. Based on the presence or absence of the Philadelphia chromosome (BCR/ABL1 translocation), MPN are broadly grouped into two categories – Philadelphia positive (chronic myeloid leukemia) and Philadelphia negative (polycythemia vera, essential thrombocythemia and myelofibrosis). The Philadelphia chromosomenegative MPNs are characterized by mutations in various genes such as JAK2, MPL, CALR, PDGFRA, PDGFRB, FGFR1 and KIT. In addition, multiple chromosome abnormalities have been defined.



DETECTION PROCESS









• **AML1-ETO Detection Kit** is intended to quantify the AML1-ETO transcripts in human bone marrow or whole blood samples of leukemia patients. The results obtained from the kit provides information about efficiency of treatment or Minimal Residual Disease (MRD) in patients.

• **PRINCIPLE**

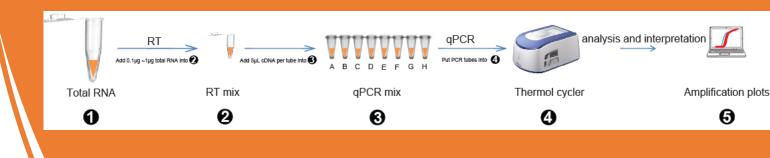
• AML1-ETO Detection Kit is a RT-qPCR based assay for quantification of AML1-ETO transcripts. The kit contains RT reaction mix and qPCR mix. cDNA is synthesized by adding purified total RNA to the RT reaction mix. The resulting cDNA is added to 2 PCR reaction tubes, which contain specific PCR primers and probes for AML1-ETO and ABL1. The qPCR is performed in a real-time thermal cycler with optical filters for FAM dye. After PCR, a linear regression curve is calculated from the standards for each gene and used for calculating the copy number of each gene in an unknown sample. AML1-ETO transcripts levels will be expressed as percentage of AML1-ETO over ABL1.



• **PML-RARα Genotyping Kit** is a qualitative in vitro diagnostic test for differentiating of PML-RARα L, PML-RARα S and PML-RARα V three splice variants with leukemia. The kit detects RNA transcripts of fusion genes extracted from human bone marrow or whole blood samples with PML-RARα. The results obtained from the kit provides more information of PML-RARα to professionals for comprehensive consideration associated with patient condition, drug indication, therapeutic response and other detection findings.

• PRINCIPLE

• **PML-RARα Genotyping Kit** is a RT-qPCR based assay for differentiating the subtype of L, S and V. Included in the kit are RT reaction mix and qPCR mix. cDNA is synthesized by adding purified total RNA to the RT reaction mix. The resulting cDNA is added to qPCR reaction tube, which contain specific PCR primers and probes for detection of fusion genes and an internal control gene of GUSB. The qPCR is performed in a real-time thermal cycler with optical filters for the detection of FAM, HEX, ROX and Cy5 fluorescence signals. Amplification plots and the resulting Cq (Quantification cycle) values are used for the identification of the fusion gene subtype. Detection of the GUSB gene is an internal control for the integrity of the RNA sample and functionality of both cDNA and qPCR reactions.







• **PML-RARα L Detection Kit** is intended to quantify the PML-RARα L transcripts in human bone marrow or whole blood samples of leukemia patients. The results obtained from the kit provides information about efficiency of treatment or Minimal Residual Disease (MRD) in patients.

• Principle

• PML-RAR α L Detection Kit is a RT-qPCR based assay for quantification of PML-RAR α L transcripts. The kit contains RT reaction mix and qPCR mix. cDNA is synthesized by adding purified total RNA to the RT reaction mix. The resulting cDNA is added to 2 PCR reaction tubes, which contain specific PCR primers and probes for PML-RAR α L and ABL1. The qPCR is performed in a real-time thermal cycler with optical filters for FAM dye. After PCR, a linear regression curve is calculated from the standards for each gene and used for calculating the copy number of each gene in an unknown sample. PML-RAR α L transcripts levels will be expressed as percentage of PML-RAR α L over ABL1.





• **PML-RARa S Detection Kit** is intended to quantify the PML-RARa S transcripts in human bone marrow or whole blood samples of leukemia patients. The results obtained from the kit provides information about efficiency of treatment or Minimal Residual Disease (MRD) in patients.

• Principle

• PML-RAR α S Detection Kit is a RT-qPCR based assay for quantification of PML-RAR α S transcripts. The kit contains RT reaction mix and qPCR mix. cDNA is synthesized by adding purified total RNA to the RT reaction mix. The resulting cDNA is added to 2 PCR reaction tubes, which contain specific PCR primers and probes for PML-RAR α S and ABL1. The qPCR is performed in a real-time thermal cycler with optical filters for FAM dye. After PCR, a linear regression curve is calculated from the standards for each gene and used for calculating the copy number of each gene in an unknown sample. PML-RAR α S over ABL1





• PML-RARα V Detection Kit is intended to quantify the PML-RARα V transcripts in human bone marrow or whole blood samples of leukemia patients. The results obtained from the kit provides information about efficiency of treatment or Minimal Residual Disease (MRD) in patients.

• Principle

• PML-RAR α V Detection Kit is a RT-qPCR based assay for quantification of PML-RAR α V transcripts. The kit contains RT reaction mix and qPCR mix. cDNA is synthesized by adding purified total RNA to the RT reaction mix. The resulting cDNA is added to 2 PCR reaction tubes, which contain specific PCR primers and probes for PML-RAR α V and ABL1. The qPCR is performed in a real-time thermal cycler with optical filters for FAM dye. After PCR, a linear regression curve is calculated from the standards for each gene and used for calculating the copy number of each gene in an unknown sample. PML-RAR α V transcripts' levels will be expressed as percentage of PML-RAR α V over ABL1





• **CBFβ-MYH11 Detection Kit** is intended to quantify the CBFβ-MYH11 transcripts in human bone marrow or whole blood samples of leukemia patients. The results obtained from the kit provides information about efficiency of treatment or Minimal Residual Disease (MRD) in patients.

• **PRINCIPLE**

• **CBF** β -**MYH11 Detection Kit** is a RT-qPCR based assay for quantification of CBF β -MYH11 transcripts. The kit contains RT reaction mix and qPCR mix. cDNA is synthesized by adding purified total RNA to the RT reaction mix. The resulting cDNA is added to 2 PCR reaction tubes, which contain specific PCR primers and probes for CBF β -MYH11 and ABL1. The qPCR is performed in a real-time thermal cycler with optical filters for FAM dye. After PCR, a linear regression curve is calculated from the standards for each gene and used for calculating the copy number of each gene in an unknown sample. CBF β -MYH11 transcripts levels will be expressed as percentage of CBF β -MYH11 over ABL1.

FDA DISCLAIMER

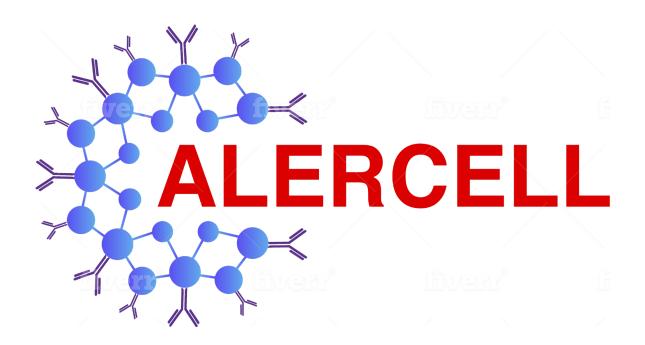
Our tests are offered to professionals only as a "RUO", For research Only and not for Diagnostics purposes.











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