This package insert must be read carefully prior to use and should be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

In Vitro Diagnosis

For Professional Use Only

INSTRUCTIONS FOR USE

PRODUCTS NAME: LENA Pan MPN qPCR Panel

PACKING: 10 tests /kit

INTENDED USE: **FOR RESEARCH USE ONLY**

LENA MPN-associated Gene Mutation Detection Kit is a specialized medical tool designed to diagnose Myeloproliferative Neoplasms (MPN), a group of diseases that affect the bone marrow and result in an abnormal increase in blood cells. This kit employs in vitro nucleic acid amplification, a laboratory technique used to increase the number of copies of a particular DNA segment, to detect mutations in three genes: JAK2, CALR, and MPL. These genes are critical in the regulation of blood cell production, and mutations in these genes are associated with the development of MPN.

The kit specifically tests for:

- **CALR Type 1/Type 2 mutations:** CALR mutations are found in certain types of MPN and are important for diagnosis, especially in cases where JAK2 mutations are not present.
- **MPL W515/S505N mutations:** Mutations in the MPL gene, including at positions W515 and S505N, can lead to abnormal blood cell growth and are indicators for certain types of MPN.
- **JAK2 V617F/K539L/exon 12 deletions:** The JAK2 gene is frequently mutated in MPN. The V617F mutation, in particular, is common in these disorders, but other mutations like K539L and deletions in exon 12 are also relevant.

By detecting these specific mutations, the LENA PAN MPN kit can help healthcare professionals diagnose MPN with greater accuracy. The results from this test are crucial for determining the presence of these gene mutations, which can significantly impact the management and treatment options for patients with MPN. It's

important to note that the kit's results should be interpreted within the context of other clinical and laboratory findings to make a comprehensive diagnosis.

The MPN-associated Gene Mutation Detection Kit is designed for utilization by healthcare professionals, including trained clinical laboratory personnel and individuals skilled in point-of-care test execution. Its application is crucial in settings requiring precision and professional competence in test administration. The integration of this kit into patient care should be holistic, taking into account not only the test results but also the patient's symptoms, medical history, additional laboratory findings, response to treatments, and relevant epidemiological data to guide clinical decision-making.

Principle of Operation

The core technology behind this kit is the Amplification Refractory Mutation System (ARMS-PCR) coupled with fluorescent probe detection. This innovative approach enables the precise identification of gene mutations related to Myeloproliferative Neoplasms (MPN) through a meticulous process:

- **Specificity through Complementary Pairing:** The technique hinges on the principle that the amplification of DNA, and thus the success of the PCR reaction, occurs only when the primer at the 3' end perfectly matches the target DNA sequence. This specificity ensures that only the DNA sequences with the mutations of interest are amplified.
- **Detection via Fluorescent Signaling:** Upon successful amplification, a fluorescent probe binds to the specific PCR product, emitting a signal that is detectable by real-time fluorescence quantitative PCR instruments. This signal, measured as cycles to threshold (Ct value), correlates with the presence of a gene mutation. A normal amplification curve indicates the presence of the mutation, while the absence of a fluorescent signal or a significantly delayed Ct value suggests a lack of the specific gene mutation.

This dual mechanism of ARMS-PCR and fluorescent probe detection provides a robust and reliable method for the identification of MPN-associated gene mutations, supporting the accurate diagnosis and management of Myeloproliferative Neoplasms. The utilization of this kit represents a sophisticated approach to genetic testing in the clinical setting, ensuring precise detection and contributing to the informed care of patients with MPN.

No.	Components	Composition	Tube	Volume (μL)
1	JAK2-V617F Mix	PCR buffer、specific primers and probes、internal control primers and probes	8-strip PCR tube	22 each tube
2	CALR TYPE1 Mix	PCR buffer、specific primers and probes、internal control primers and probes	8-strip PCR tube	22 each tube
3	CALR TYPE2 Mix	PCR buffer、specific primers and probes、internal control primers and probes	8-strip PCR tube	22 each tube
4	MPL-W515 Mix	PCR buffer、specific primers and probes、internal control primers and probes	8-strip PCR tube	22 each tube
5	MPL-S505N Mix	PCR buffer、specific primers and probes、internal control primers and probes	8-strip PCR tube	22 each tube
6	JAK2-K539L Mix	PCR buffer、specific primers and probes、internal control primers and probes	8-strip PCR tube	22 each tube
7	JAK2 e12 del Mix	PCR buffer、specific primers and probes、internal control primers and probes	8-strip PCR tube	22 each tube
8	Enzyme Mix	Hot-start Taq DNA Polymerase、Uracil-DNA Glycocasylase	Red cap tube	96
9	Positive control	Plasmids and salmon sperm DNA	Green cap tube	96
10	No-template control	Salmon sperm DNA	Blue cap tube	96

The distribution of each PCR reaction mix in the 8-strip PCR tube is as follows (the numbered end of the 8-strip PCR tube is at the front and the empty reaction tube is at the last:

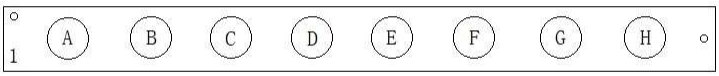

Fig. 1. Distribution of each PCR reaction mix in the 8-strip PCR tube

A: JAK2-V617F Mix; B: CALR TYPE1 Mix; C: CALR TYPE2 Mix; D: MPL-W515 Mix; E: MPL-S505N Mix; F: JAK2-K539L Mix; G: JAK2 e12 del Mix; H: Empty reaction tube.

Note: The components of the kits from different batch cannot be interchangeable.

Additional required material (not included in the test kit)

Reagent & consumables	Recommend	Use
DNA extraction reagent	QIAamp DNA Blood Mini Kit (QIAGEN, Cat. No. 51104)	DNA extraction
TE buffer or Ultra-pure water	NA	Dilution

Other common experimental consumables such as pipette tips and centrifuge tubes shall be provided by the user.

Storage

Store at -20±5 °C without light and is valid for 12 months. See production date and expiry date on the label.

Applicable instruments

Applied Biosystem 7500 Real-Time PCR System (ABI 7500), Hongshi SLAN®-96P Real-Time PCR System (SLAN-96P)

Sample requirement

1. EDTA Human whole blood.
2. Sample quality control requirements:
    1) 5ng/μl ≤ DNA concentration ≤ 10ng/μl；
    2) OD260/OD280 ratio between 1.7 and 2.0;
    3) DNA samples are stored at-20±5℃ for no more than 6 months

Methodology

1. DNA Extraction

    Human genomic DNA was extracted according to the instruction of the DNA extraction kit.

    The concentration and OD260nm/OD280nm ratio of DNA should be determined by a spectrophotometer (ThermoFisher Nanodrop One is recommended). The concentration should be 5-10 ng/μL. If the concentration is higher, the DNA should be diluted; if the concentration is lower, DNA should be extracted again. The OD260nm/OD280nm ratio should be 1.7-2.0. If the OD260nm/OD280nm ratio is out of range, DNA should be extracted again.

    If DNA cannot be detected immediately, it should be stored at -20℃±5℃ for no more than 6 months.

2. Reaction preparation

    The kit does not need to prepare a master mix, and the PCR mix has been distributed into the 8-strip PCR tube. Please mix reagent well and centrifuge before.

In this kit, DNA samples and the enzyme mix need to be pre-mixed, mixed and briefly centrifuged after being pre-mixed, and add 3μl mixture into the 8-strip PCR tube.

Table 1. DNA samples pre-mixed with enzyme mix

DNA Sample volume (1×)	Enzyme mix volume (1×)
16μl	8μl

Adding 3μl mixture according to the following table. Positive control and no-template control should be tested as experimental quality control. The controls also need to be pre-mixed according to the above table with the enzyme mix.

After adding the mixture, cover the 8-strip tube covers, and ensure that the end with numbers on the tube covers is coincident with the end with numbers on the tube body, so as to reduce the pollution between different PCR mixes.

Table 2. Plate setup

(It is recommended that the numbered end of the 8-strip PCR tube is at the front and the empty reaction tube is at the last)

Mutations		1	2	3	4	5	6	7	8	9	10	11	12
JAK2 V617F	A	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	PC	NTC
CALR TYPE1	B	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	PC	NTC
CALR TYPE2	C	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	PC	NTC
MPL W515	D	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	PC	NTC
MPL S505N	E	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	PC	NTC
JAK2 K539L	F	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	PC	NTC
JAK2 e12 del	G	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	PC	NTC
	H												

Note: S1-S10: DNA sample 1-10; PC: Positive control; NTC: No-template control

3. PCR program

Table 3. PCR program

Mode of analysis			Standard Curve – Absolute Quantitation
Steps	Temperature	Time	Note
1	50℃	2min	去污染
2	95℃	3min	Hold2: Predegeneration
3	95℃	15s	Cycling: 45 times ※: Acquisition of fluorescence Fluorescence: FAM, VIC Note: Passive reference select “None” when using ABI 7500
	60℃	1min※	

4. Mix well and centrifuge, put the PCR tube into the PCR instrument and run the

PCR program.

5. When the PCR program is finished, run the analysis software:

1） ABI 7500: Click “Analysis Settings”, setting the threshold value manually, the corresponding Ct values of each reaction well can be viewed in the “View Well Table”, and the Ct values of different reaction wells are marked as Ct (FAM) and Ct (VIC) respectively.

2）SLAN-96P: Click “Analysis”, setting the threshold value manually, the corresponding Ct values of each reaction well can be viewed in the “Information table of reaction wells”, and the Ct values of different reaction wells are marked as Ct (FAM) and Ct (VIC) respectively.

3） The mutation detection signal is FAM and the internal control signal is VIC.

Requirements for results analysis

1. Requirements for baseline:

Use ABI 7500 and Hongshi slan-96p instrument default baseline.

2. Requirements for threshold:

ABI 7500: The threshold of the mutation detection signal (FAM) is 75000, and the internal control signal (VIC) is 25000.

SLAN-96P: The threshold of the mutation detection signal (FAM) is 0.08, and the internal control signal (VIC) is 0.08.

3. Cut-off value:

ABI 7500:

If the Ct (VIC) of the internal control signal is ≤ 35, the mutation detection signal has amplification (FAM channel, typical "S" curve), and the Ct (FAM) is ≤ 40, then the detection result of mutations of CALR-TYPE1/TYPE2, MPL-W515/S505N, JAK2-K539L/e12 del is positive; the Ct (FAM) is ≤ 39.51, then the detection result of mutations of JAK2-V617F is positive.

If Ct (FAM) is > 40 or "Undetermined" or no amplification curve, the detection result of mutations of CALR-TYPE1/TYPE2, MPL-W515/S505N, JAK2-K539L/e12 del is negative; Ct (FAM) is > 39.51 or "Undetermined" or no amplification curve, the detection result of mutations of JAK2-V617F is negative.

SLAN-96P:

If the Ct (VIC) of the internal control signal is ≤ 35, the mutation detection signal has amplification (FAM channel, typical "S" curve), and the Ct (FAM) is ≤ 40, then the detection result of mutations of CALR-TYPE1/TYPE2, MPL-W515/S505N, JAK2-K539L/e12 del is positive; the Ct (FAM) is ≤ 38.55, then the detection result of mutations of JAK2-V617F is positive.

If Ct (FAM) is > 40 or "Undetermined" or no amplification curve, the detection result of mutations of CALR-TYPE1/TYPE2, MPL-W515/S505N, JAK2-K539L/e12 del is negative; Ct (FAM) is > 38.55 or "Undetermined" or no amplification curve, the detection result of mutations of JAK2-V617F is negative.

Interpretation of results

1. Controls: If all controls meet the criteria listed in Table 4-1 or 4-2 and samples are tested in the same PCR reaction, the PCR reaction is valid and the results can be

analyzed further.

Table 4-1. Controls criteria (ABI 7500)

Controls	Ct (FAM)	Ct (VIC)
Positive control	Ct≤35	Ct≤35
No-template control	“Undetermined” or Ct＞40	“Undetermined”or Ct＞40

Table 4-2. Controls criteria (SLAN-96P)

Controls	Ct (FAM)	Ct (VIC)
Positive control	Ct≤35	Ct≤35
No-template control	“Undetermined” or Ct＞40	“Undetermined”or Ct＞40

2. If the results of positive control is not in the range or there is no fluorescence signal or no obvious amplification curve (even if Ct value is detected), the reaction is fail, and repeated testing or contact the manufacturer.

3. If No-template control has an amplification curve and the Ct > 40, there is no effect on the results; If the Ct ≤ 40, it indicates that there is contamination in the PCR reaction, please repeat experiment.

4. The interpretation of the results of tested samples according to the Table 5-1, Table 5-2 or Table 5-3, Table 5-4.

Table 5-1. Interpretation of the results of tested samples (ABI 7500)

Results of JAK2-V617F	Ct(FAM)	Ct(VIC)
Positive	Ct ≤ 39.51	Ct ≤ 35
Negative	Ct > 39.51 or "Undetermined" or no amplification curve	Ct ≤ 35
Invalid	Any condition	Ct > 35 or "Undetermined" or no amplification curve

Table 5-2. Interpretation of the results of tested samples (ABI 7500)

Results of CALR-TYPE1/TYPE2 or MPL-W515/S505N or JAK2-K539L/e12 del	Ct(FAM)	Ct(VIC)
Positive	Ct ≤ 40	Ct ≤ 35
Negative	Ct > 40 or "Undetermined" or no amplification curve	Ct ≤ 35
Invalid	Any condition	Ct > 35 or "Undetermined" or no amplification curve

Table 5-3. Interpretation of the results of tested samples (SLAN-96P)

Results of JAK2-V617F	Ct(FAM)	Ct(VIC)

Positive	Ct ≤ 38.55	Ct ≤ 35
Negative	Ct > 38.55 or "Undetermined" or no amplification curve	Ct ≤ 35
Invalid	Any condition	Ct > 35 or "Undetermined" or no amplification curve

Table 5-4. Interpretation of the results of tested samples (SLAN-96P)

Results of CALR-TYPE1/TYPE2 or MPL-W515/S505N or JAK2-K539L/e12 del	Ct(FAM)	Ct(VIC)
Positive	Ct ≤ 40	Ct ≤ 35
Negative	Ct > 40 or "Undetermined" or no amplification curve	Ct ≤ 35
Invalid	Any condition	Ct > 35 or "Undetermined" or no amplification curve

5. If the results of samples are not in the range or there is no fluorescence signal or no obvious amplification curve (even if Ct value is detected), the reaction is fail, and repeated testing or contact the manufacturer.

6. If the internal control signal of the sample is amplified, but the Ct value (VIC) is > 35, it indicates that the concentration of the DNA is too low, it is necessary to re-extract the DNA and dilute it to the range of 5 ~ 10 ng/μL; If the problem still exists, contact the manufacturer.

Limitations

1. This kit cannot be used to test whole blood and other tissue samples directly.

2. The concentration requirement of DNA is 5 ~ 10 ng/μL.

3. The test results of this kit cannot be used as the sole basis for clinical diagnosis. The clinical management of patients should be considered in combination with their symptoms/signs, medical history, other laboratory tests, treatment response, epidemiology and other information.

Performance characteristics

1. Appearance and properties

   All reaction mixes are lilac transparent clarified liquid, free of precipitate, suspended solids and flocs.

   The enzyme mix is a transparent viscous liquid without precipitation, suspended matter and floc;

   The positive control is transparent liquid without precipitation, suspended matter and floc.

   The no-template control is transparent liquid without precipitation, suspended matter

and floc.

2. Accuracy

   Test the strong and weak positive standards once, the result should be positive.

   Test the negative standard once, the result should be negative.

3. Stability

   Test the kit that have expired (within 2 months after expiration date), the result should meet item 1.-2.

[Notice]

1. Upon arrival of the kit, check whether the outer package is complete and take out the positive control and place it in the specimen preparation area, and store it at -20℃±5℃.
2. Experimental personnel must be professionally trained and qualified for PCR detection, and should read this instruction carefully before testing.
3. The relevant laboratory management standards shall be strictly implemented in accordance with the management standards for gene amplification testing laboratories issued by the competent administrative departments of the industry.
4. The laboratory should be operated in strict zones (reagent preparation area, sample preparation area, amplification and product analysis area). The consumables used should be PCR-grade and disposable. Experimental wastes shall be disposed in accordance with relevant laboratory management regulations.
5. When dividing the PCR reaction mix, bubbles should be avoided as far as possible. Before loading the machine, pay attention to check whether the reaction tubes are tightly covered, so as not to leak fluorescent substances and pollute the instrument.
6. When adding sample, the sample should be completely added into the reaction solution, and the sample should not be attached to the tube wall. After adding sample, the tube cover should be closed as soon as possible.
7. This kit has high requirements on the quality of DNA to be tested, and the DNA to be tested must meet the requirements of the instructions.
8. Reagents of different batches should not be mixed, and should be used within the validity period.
9. Immediately take out the PCR tube after amplification and seal it in a special plastic bag.
10. This kit is only used for in vitro testing. Interpretation of test results should be combined with other clinical diagnostic information.
11. This kit is not suitable for people with a history of blood transfusion and bone marrow transplantation.

[Reference]

1. Guidelines for the Preparation of Instructions for In Vitro Diagnostic Reagents (No. 17 of 2014)
2. Regulations on the Management of Medical Device User manual and Labels (Decree No. 6)
3. Guidelinesfor the Technical Review of Reagentsfor the Detection of Mutations in Genes Associated with Personalized Cancer Therapy (issued 2014-03-13)
4. A Tefferi et al. Leukemia (2008) 22, 14–22
5. Daniel A. Arber et al. BLOOD, 19 MAY 2016 VOLUME 127, NUMBER 20

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Explanation of symbols:

	CE mark		Batch code
	Consult Instructions for use		In-vitro Medical Device
	Use-by date		Date of manufacture
	Temperature limit		Do not use if package is damaged
	manufacturer		Authorized representative in the European community
	Keep dry		Can't be reused