Формула гена

MYELOSCREEN

the kit for detection of driver mutations in chronic myeloproliferative neoplasia by real-time multiplex PCR

mutation	gene
V617F	JAK2
c.1092_1143del (p.L367fs*46)	CALR
c.1154_1155insTTGTC (p.K385fs*47)	CALR
W515K	MPL
W515L	MPL

Instructions for use

1. DESTINATION

The Myeloscreen reagent kit is designed for simultaneous quantitative determination of the V617F mutation in the Janus kinase-2 (JAK2) gene, type 1 and type 2 mutations in the CALR gene, and W515L, W515K mutations in the MPL gene by real-time PCR. The analyzed sample is DNA isolated from whole blood leukocytes or dried blood spots.

2. DESCRIPTION OF THE KIT

2.1 Principle of the method

The method for detecting the V617F mutation in the JAK2 gene, type 1 and type 2 mutations in the CALR gene, W515L, W515K mutations in the MPL gene is based on nucleic acid amplification with real-time fluorescent signal detection using specific TaqMan probes. The amplification result is registered through the FAM, HEX/JOY and ROX fluorescence channels. The wild-type allele of the JAK2 gene is used as an internal control.

To perform the analysis, PCR is required in two tubes per patient:

Mix 1: Wild-type JAK2 allele (FAM channel), c.1092_1143del mutant CALR gene allele (HEX/JOY channel), W515K mutant MPL gene allele (ROX channel).

Mix 2: Mutant-type JAK2 allele (FAM channel), mutant allele of CALR gene with c.1154_1155insTTGTC mutation (HEX/JOY channel), mutant allele of MPL gene with W515L mutation (ROX channel).

- Fluorescent signal detection threshold (Ct) is determined for all tubes

(automatic Ct calculation is recommended).

- The presence or absence of a mutation is determined by the value of Ct (see **Analysis and reporting of results**).
- *PCS* (positive control sample) is a vector with DNA segments carrying mutant alleles of all genes under study.
- *NCS* (negative control sample) is a vector with a DNA region that does not have mutations in the studied genes.

2.2 Composition of the kit

The kit is designed for 50 tests, including positive and negative controls. The kit is supplied in a plastic container. All reagents are packaged in sealed plastic vials with color-coded labels and caps. All components of the kit are ready to use, there are no lyophilized components in the kit.

The kit contains:

Reagent	Description	Total volume, µl	Amount, tubes
Mix 1	Colorless transparent liquid	900	1
Mix 2	Colorless transparent liquid	900	1
Taq-polymerase	Colorless transparent liquid	65	1
PCS (positive control sample)	Colorless transparent liquid	200	1
NCS (negative control sample)	Colorless transparent liquid	200	1
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3. ANALYTICAL CHARACTERISTICS

Internal control. Each kit is supplied with an internal control, which is a mixture containing primers specific for the wild-type allele of the JAK2 gene.

Sensitivity. The analytical sensitivity of the method is 0.5% of the relative concentration of the V617F mutation in the JAK2 gene, 3% of the relative concentration of type 1 and type 2 mutations in the gene

CALR, 3% relative concentration of W515L and W515K mutations in the MPL gene in a clinical sample.

Specificity. The analysis reliably detects only V617F mutations in the JAK2 gene, W515K and W515L mutations in the MPL gene, type 1 and 2 mutations in the CALR gene. The frequency of detection of mutations based on the results of testing more than 6 thousand patients with suspected MPN is fully consistent with the literature data. Low levels (less than 2%) of the JAK2 V617F allele burden can be detected in blood donors (with a frequency of up to 0.8%), as well as in elderly patients with cardiovascular diseases and ischemic strokes (with a frequency of up to 5%) with the syndrome of "clonal hematopoiesis of uncertain potential" or "CHIP syndrome").

4. PRECAUTIONS

1. The kit does not contain substances and materials that require special safety measures, and does not pose a danger to people during the entire shelf life as well as all routine precautions when handling patient biological specimens.

5. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

1. Real-time PCR thermal cycler.
2. Nucleic acid extraction kit.
3. Vortex mixer.
4. UV PCR cabinet.
5. Freezer.
6. Refrigerator.
7. Single channel pipettes (dispensers covering 20-1000 µL volume range).
8. RNase and DNase free filtered pipette tips (volume 20 µL, 50 µL, 200 µL, 1000 µL).
9. Отдельный халат и одноразовые резиновые перчатки.
10. Container for used pipette tips, tubes and other consumables.
11. Powder-free surgical gloves.
12. 0.2 mL tubes.
13. 1.5 mL tubes.
14. PCR tube rack for 0.2 mL tubes.

6. SAMPLES FOR ANALYSIS

Genomic DNA isolated from peripheral blood or bone marrow should be used as a sample. It is possible to use DNA isolated from dried blood spots (DBS) collected on FTA cards.

7. CONDITIONS FOR TRANSPORTATION, STORAGE AND USE

Transportation of the kit must be done in thermal containers with ice packs, avoiding long-term (more than 12 hours) defrosting. Multiple

(more than 20 cycles) freezing-thawing should be avoided. Transportation of a reagent kit can be carried out by all types of covered transport, subject to the necessary temperature conditions in accordance with the rules established for this type of transport. Storage of kit should be carried out in the manufacturer's packaging in a dry, dark place in compliance with the required temperature conditions.

8. EXPIRY DATE

The expiration date of the kit is 12 months from the date of production, subject to the conditions of transportation and storage. Do not use the kit after the set expiration date. The expiration date and storage conditions for opened reagents correspond to the expiration date and storage conditions indicated on the label for unopened reagents.

9. PERSONNEL REQUIREMENTS

The kit is intended for professional use and must be used by trained personnel experienced in performing PCR tests.

10. PROTOCOL OF ANALYSIS

10.1 Preparation of reagents

All kit components are ready to use.

10.2 Test procedure

1. Defrost the kit components at room temperature, **except for Taq polymerase (!)**, mix on a vortex. Centrifuge for 5 seconds at 5000 rpm in a microcentrifuge to remove drops from the caps of the tubes.

2. Prepare the required volumes of the reactions mix for N samples according to Table 3.
3. The calculation includes the productions of PCS and NCS. Vortex thoroughly. Centrifuge for 5 seconds at 5000 rpm in a microcentrifuge to remove drops from the caps of the tubes.

Table 3. Calculation of the required amount of PCR mixtures for the analysis of N-samples

Mix	**Volume, µl**	**Taq-polymerase, µl**
Mix 1	(N+2)*15	(N+2)*0,5
Mix 2	(N+2)*15	(N+2)*0,5

Taq polymerase must be used directly from the freezer. After adding to the reaction mixtures, immediately place in the freezer.

4. Select the required number of PCR-tubes. The calculation of the number of PCR-tubes is carried out since each sample is examined in two PCR mixtures (“Mix 1”, “Mix 2”), and in addition, PCS and NCS (Table 3) for each mix.
5. Add 15 µl of the appropriate reaction mix to each PCR-tube.
6. Defrost test samples, PCS and NCS.
7. Add 10 µl of patient DNA samples (recommended of 20-100 ng), PCS and NCS to test tubes with reaction mix using tips with an aerosol barrier. Vortex the tubes for 3-5 seconds.
8. Set the tubes into the Real-time Thermal Cycler. Install the program for amplification and detection of the fluorescent signal (see Table 4).

Table 4. PCR amplification protocol

Cycle	Step	Number of cycles	Time	Temperature	Camera
1	1	1	5 min	95°C	
2	1	50	15 sec	95°C	
	2		60 sec	60°C	On (FAM, HEX/JOY, ROX)

Set up the following fluorescence reading channels:

Mix 1:

- FAM – JAK2 wild-type;
- HEX/JOY – mutation c.1092_1143del in the CALR gene;
- ROX - W515K mutation in the MPL gene.

Mix 2:

- FAM - V617F mutation in the JAK2 gene;
- HEX/JOY – mutation c.1154_1155insTTGTC in the CALR gene;
- ROX - W515L mutation in the MPL gene.

10.3 Analysis and reporting of results

The data obtained - the curves of the accumulation of the fluorescent signal are analyzed using the software of the device used for PCR in the "real time" mode in accordance with the instructions for the device.

1. Determine the threshold cycle (Ct) for each tube. Automatic calculation of the threshold cycle is recommended.
2. Threshold cycles of the NCS must be greater than 35*, otherwise

the results of all test samples are considered unreliable.

3. The threshold cycle of all tubes of the PCS must be less than 35*, otherwise the results of all the studied samples are considered unreliable.
4. For all samples under study, the threshold cycle of **mix 1** on the FAM channel must be less than 30*. Failure to comply with this condition indicates a small amount of DNA added to the sample. It is necessary to repeat the examination of the clinical sample from the extraction stage. Possible reasons: an error in the processing of clinical material, which led to the loss of DNA, or inhibition of PCR.
5. Threshold cycles of all tubes should not be less than 15*. Failure to comply with this condition indicates an excess amount of DNA added to the sample. It is necessary to repeat the analysis with preliminary dilution of DNA.
6. To calculate the allele burden of the V617F mutation, use the formula:

$$V617F, \% = \frac{100}{1 + 2^{Ct\ FAM(mix\ 2) - Ct\ FAM(mix\ 1)}}$$

If the allele burden value is > 0.5%, the sample is considered positive for the V617F JAK2 mutation.

7. To determine the presence of mutations in the CALR gene, use the formulas:

$$CALR\ \text{c.}1092\_1143\text{del} = \frac{100}{1 + 2^{Ct\ HEX(mix\ 1) - Ct\ FAM(mix\ 1)}}$$

If the value of CALR c.1092_1143del > 3, the sample is considered

positive for the c.1092_1143del mutation of the CALR gene.

$$CALR\ \text{c.}1154\_1155\text{insTTGTC} = \frac{100}{1 + 2^{Ct\ HEX(mix\ 2) - Ct\ FAM(mix\ 1)}}$$

If the value c.1154_1155insTTGTC > 3, the sample is considered positive for the c.1154_1155insTTGTC mutation of the CALR gene.

8. The presence of the W515K mutation in the MPL gene is determined at Ct < 35 mix 1 for the ROX channel. The presence of the W515L mutation in the MPL gene is determined at Ct < 35 of mix 2 for the ROX channel
9. In rare cases (in 2-4%), patient samples may contain several mutations at the same time.

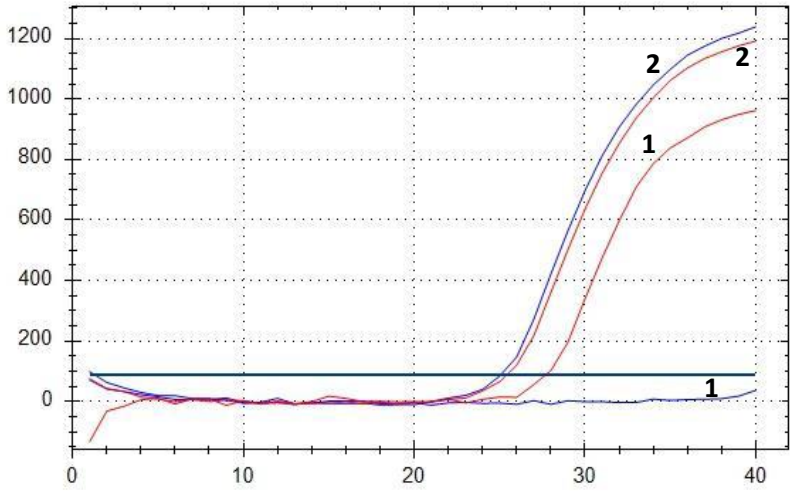

Figure. Curves example

An example of fluorescent signal accumulation curves in samples with JAK2 V617F mutation and without JAK2 V617F mutation: the patient with JAK2 V617F mutation is shown in red, the patient without JAK2 V617F mutation is in blue; 1 – mix 1, FAM channel; 2 – mix 2, FAM channel.

11. LIMITATIONS

This test gives quantitative results and shows the presence of mutations in the sample, which, however, should not be considered as the only criterion for the diagnosis of MPN. As with any other diagnostic testing, all results must be interpreted in conjunction with other laboratory and clinical findings.

12. DISPOSAL (DESTRUCTION) AND DISINFECTION

When handling test specimens and waste, the precautions for handling potentially infectious material should be followed.

13. MANUFACTURER WARRANTY

The manufacturer guarantees the compliance of the set with the requirements, subject to the conditions of transportation, storage and use.

For questions about the quality of the kit, please contact Formula of gene LLC at the address: 660036, Russia, Krasnoyarsk, st. Akademgorodok, 50/45, office 102, tel. +7(391)290-55-13, e-mail: mail@formulagena.ru.

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