

MPL W515L/K MUTATION (MYELOPROLIFERATIVE LEUKEMIA VIRUS ONCOGENE)

ORDERING INFORMATIONS

REF: *ONC-013-25 RDM Code: 1772905/R*
Tests: *25 Reactions: 31 x 2*
REF: *ONC-013-50 RDM Code: 2256722/R*
Tests: *50 Reactions: 62 x 2*
CND Code: *W01060299*
Manufacturer: *BioMol Laboratories s.r.l.*

CONTENTS OF THE KIT

The kit consists of: reagents for Real-Time PCR amplification
**the reagents for the extraction of genomic DNA are not supplied in the kit*

For in vitro diagnostic use



PRODUCT CHARACTERISTICS

Device belonging to the family of in vitro medical devices **REAL-TIME QUALITATIVE PCR-SOMATIC MUTATIONS**. Detection of the W515L/K mutation of the MPL gene (MYELOPROLIFERATIVE LEUKEMIA VIRUS ONCOGENE) by Real-Time PCR technique. Kit optimized for Real Time PCR instrumentation Biorad CFX96, Biorad Opus Dx, Agilent AriaDx, Hyris bCUBE and Hyris bCUBE3 with Hyris bAPP.

SCIENTIFIC BACKGROUND

Myeloproliferative neoplasms (MPNs) are hematological malignancies characterized by the proliferation of one or more myeloid lineages: granulocytic, erythroid, megakaryocytic and/or mast cell. The JAK (Janus Kinases) family of enzymes includes JAK1, JAK2, JAK3, and TYK2. These molecules bind to the cytosolic domains of cytokine receptors and are essential for the transduction of cytokine and growth factor messages. Polycythemia vera (PV), idiopathic myelofibrosis (PMF) and essential thrombocythemia (ET) show shared phenotypic features (MPN BCR/ABL neg) that are the consequence of direct or indirect constitutive activation of JAK2, the tyrosine kinase related to hematopoietic growth factor receptors for erythropoietin (EPOR) and thrombopoietin (MPL) and to the G-CSF receptor (Granulocyte Colony-Stimulating Factor).

CLINICAL SIGNIFICANCE

Direct activation of JAK2 is caused by a point mutation (V617F in exon 14 JAK2 or, less commonly, by insertions or deletions in exon 12 of the JAK2 gene). Indirect activation, on the other hand, is caused by point mutations in the thrombopoietin receptor, MPL or by mutations in the CALR chaperone calreticulin (CALR) gene that allow MPL to bind and activate JAK2 indirectly. The JAK2 V617F mutation is the result of the substitution of a guanine in thymine at nucleotide 1849 of exon 14 of the JAK2 gene, which causes a single valine/phenylalanine amino acid substitution at codon 617. The mutation causes ligand-independent JAK2 kinase activity. This mutation can be found in about 70% of Philadelphia chromosome-negative MPNs (Ph-MPDs); it is present in 65-95% of PV patients, 23-57% of ET patients and 35-50% of PMF patients. The new molecular knowledge in the field of chromosome-negative Philadelphia MPNs has made it possible to identify the V617F mutation of the JAK2 gene as a safe diagnostic criterion to be included in the laboratory routine in case of suspected MPN (as suggested by the diagnostic criteria of the WHO (World Health Organization; Tefferi et al. Leukemia 2008).

§ The 2016 WHO classification and diagnostic criteria for myeloproliferative neoplasms: document summary and in-depth discussion. Blood Cancer J.2018 Feb 9; 8 (2):15. Doi: 10.1038/s41408-018-0054-y. Review

§ Essential thrombocythemia: a review of the clinical features, diagnostic challenges, and treatment modalities in the era of molecular discovery. Leuk Lymphoma. 2017 Dec; 58 (12):2786-2798. doi: 10.1080/10428194.2017.1312371. Epub 2017 May 15.

§ Genetic basis and molecular pathophysiology of classical myeloproliferative neoplasms. Blood. 2017 Feb 9; 129 (6):667-679. Review.

§ Mutations in MPNs: prognostic implications, window to biology, and impact on treatment decision. Hematology Am Soc Hematol Educ Program.2016 Dec 2; 2016 (1):552-560.

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DESCRIPTION	LABEL	VOLUME		STORAGE
		ONC-013-25	ONC-013-50	
Mix oligonucleotides and probes	Mix W515L MPL 10 X	1 x 77,5 µl	2 x 77,5 µl	- 20 °C
Mix oligonucleotides and probes	Mix W515K MPL 10 X	1 x 77,5 µl	2 x 77,5 µl	- 20 °C
Mix buffer and Taq-polymerase	Mix Real-Time PCR 2X	1 x 775 µl	2 x 775 µl	- 20 °C
Deionized H ₂ O	Deionized H ₂ O	1 x 1 ml	1 x 1 ml	- 20 °C
Genomic DNA or recombinant DNA Negative control	Negative control W515W MPL	1 x 40 µl	2 x 40 µl	- 20 °C
Genomic DNA or recombinant DNA Positive control	Positive control W515L MPL W515K MPL	1 x 40 µl	2 x 40 µl	- 20 °C

TECHNICAL CHARACTERISTICS

COD. ONC-013-25 / COD. ONC-013-50

STABILITY	18 months
REAGENTS STATUS	Ready to use
BIOLOGICAL MATRIX	Genomic DNA extracted from whole blood, tissue, cells
POSITIVE CONTROLS	Recombinant DNA for at least 3 analytical sessions (ONC-013-25) Recombinant DNA for at least 6 analytical sessions (ONC-013-50)
TECHNOLOGY	Real-time PCR; oligonucleotides and specific probes; 2 FAM/HEX fluorescence channels
VALIDATED INSTRUMENTS	Biorad CFX96 Dx, Biorad Opus Dx, Agilent AriaDx, Hyris bCUBE and Hyris bCUBE3 with Hyris bAPP.
RUNNING TIME	110 min
THERMAL CYCLING PROFILE	1 cycle at 95 °C (10 min); 50 cycles at 95 °C (15 sec) + 60 °C (1 min)
ANALYTICAL SPECIFICITY	Absence of non-specific pairings of oligonucleotides and probes; absence of cross-reactivity
LIMIT OF DETECTION (LOD)	≥ 0,025 ng of genomic DNA, ≥ 2%
LIMIT OF BLANK (LOB)	0% NCN
REPRODUCIBILITY	99,9%
DIAGNOSTIC SPECIFICITY / DIAGNOSTIC SENSITIVITY	100%/98%