

CALR EXON 9 MUTATION

Type I (DEL 52bp) And Type II (INS 5bp) (chaperone calreticulin)

ORDERING INFORMATIONS

REF: *ONC-014-25 RDM Code: 1761183/R*
Tests: *25 Reactions: 31 x 2*
REF: *ONC-014-50 RDM Code: 2256763/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**. Qualitative detection of the INS 5bp/DEL 52bp mutation of exon 9 of the CALR gene (chaperone calreticulin) by Real-Time PCR technique. The kit is optimized for Real-Time PCR instruments Biorad CFX96 Dx, 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. According to the 2016 World Health Organization criteria, the classification of MPNs includes seven subcategories: chronic myeloid leukemia (CML), chronic neutrophilic leukemia, polycythemia vera (PV), primary myelofibrosis (PMF), essential thrombocythemia (ET), chronic eosinophilic leukemia - not otherwise specified, and MPN, unclassifiable (MPN-U). 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). 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 of JAK2 is caused by point mutations in the thrombopoietin receptor, MPL or by mutations in the CAL chaperone calreticulin (CALR) gene that allow MPL to bind and activate JAK2 indirectly. CALR is a multi-functional protein (Ca²⁺-binding protein) with chaperone activity, mainly located in the endoplasmic reticulum (ER).

§ *Cancers (Basel). 2024 Apr 26;16(9):1679. doi: 10.3390/cancers16091679. Advances in Molecular Understanding of Polycythemia Vera, Essential Thrombocythemia, and Primary Myelofibrosis: Towards Precision Medicine*
§ *Front. Cell Dev. Biol., 26 March 2024 Sec. Cancer Cell Biology Volume 12 – 2024*
§ *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. Review*
§ *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.*
§ *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.*

CLINICAL SIGNIFICANCE

Somatic mutations of CALR are often represented by deletions/insertions in exon 9 and generate a "frameshift" mutation on the reading frame resulting in a new amino acid sequence at the carboxy-terminal domain of the protein. The mutated protein also loses the KDEL signal, which is necessary for the protein to localize in the endoplasmic reticulum. The two most frequent mutations correspond to a deletion of 52 bp (p.L367fs*46), also called type 1, and an insertion of 5 bp (p.K385fs*47), also called type 2. CALR mutations usually occur in the heterozygous state although few cases of homozygous mutations have been observed, more often for type 2 mutations.

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DESCRIPTION	LABEL	VOLUME		STORAGE
		ONC-014-25	ONC-014-50	
Mix oligonucleotides and probes	Mix Ins 5bp CALR 10X	1 x 77,5 µl	2 x 77,5 µl	- 20 °C
Mix oligonucleotides and probes	Mix Del 52bp CALR 10X	1 x 77,5 µl	2 x 77,5 µl	- 20 °C
Mix buffer and Taq-polymerase	Mix Real-Time PCR 5X	1 x 310 µl	2 x 310 µl	- 20 °C
Deionized H ₂ O	Deionized H ₂ O	1 x 1 ml	2 x 1 ml	- 20 °C
Genomic DNA or recombinant DNA Positive control	Positive control Ins 5bp CALR Del 52bp CALR	1 x 30 µl	2 x 30 µl	- 20 °C
Genomic DNA or recombinant DNA Negative Control	Negative control	1 x 30 µl	2 x 30 µl	- 20 °C

TECHNICAL CHARACTERISTICS

COD. ONC-014-25 / COD. ONC-014-50

STABILITY	18 months
REAGENTS STATUS	Ready to use
BIOLOGICAL MATRIX	Genomic DNA extracted from whole blood, tissues, cells
POSITIVE CONTROLS	Recombinant DNA for at least 3 analytical sessions (ONC-014-25) Recombinant DNA for at least 6 analytical sessions (ONC-014-50)
NEGATIVE CONTROLS	Recombinant DNA for at least 3 analytical sessions (ONC-014-25) Recombinant DNA for at least 6 analytical sessions (ONC-014-50)
TECHNOLOGY	Real-time PCR; oligonucleotides and specific probes for mutations and internal amplification control; 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, ≥ 1%
LIMIT OF BLANK (LOB)	0% NCN
REPRODUCIBILITY	99,9%
DIAGNOSTIC SPECIFICITY / DIAGNOSTIC SENSITIVITY	100%/98%