CALR (chaperone calreticulin) EXON 9 Type I (Del 52bp) AND Type II (Ins 5bp) MUTATION

For in vitro diagnostic use

CE IVD

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*

PRODUCT CHARACTERISTICS

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 hematologic 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 MPN classification includes seven subcategories: chronic myeloid leukemia (CML), chronic neutrophilic leukemia, polycythemia vera (PV), primary myelofibrosis (PMF), essential thrombocythemia (ET), eosinophilic leukemia chronic - 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 result from direct or indirect constitutive activation of the related tyrosine kinase JAK2 to the hematopoietic growth factor receptors for erythropoietin (EPOR) and thrombopoietin (MPL) and to the G-CSF (granulocyte colony-stimulating factor) receptor. Direct activation of JAK2 is caused by a point mutation (V617F in JAK2 exon 14) 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 localized in the endoplasmic reticulum (ER).

§ 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.

§ 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

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. Furthermore, the mutant protein loses the KDEL signal, necessary for the localization of the protein in the endoplasmic reticulum. The two most frequent mutations correspond to a 52 bp deletion (p.L367fs*46), also called type 1, and a 5 bp insertion (p.K385fs*47), also called type 2. CALR mutations usually occur at the heterozygous state although few cases of mutations in the homozygous state have been observed, more often for type 2 mutations.

BIOMOL LABORATORIES S.R.L.
Via Arcora 110 (Palazzo Gecos)
80013 Casalnuovo di Napoli, NA
info@biomollaboratories.com
biomollaboratories.it

ISO 9001:2015
ISO 13485:2016

BioMol LABORATORIES

CALR (chaperone calreticulin) EXON 9 Type I (Del 52bp) AND Type II (Ins 5bp) MUTATION

For in vitro diagnostic use

CE IVD

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 srl

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*

CONTENTS OF THE KIT

DESCRIPTION	LABEL	VOLUME		STORAGE
		ONC-014-25	ONC-014-50	
Mix oligonucleotides and probes	Mix Ins 5bp CALR 10X	1 x 85 µl	1 x 170 µl	- 20 °C
Mix oligonucleotides and probes	Mix Del 52bp CALR 10X	1 x 85 µl	1 x 170 µl	- 20 °C
Mix buffer and Taq-polymerase	Mix Real-Time PCR 5X	1 x 350 µl	1 x 700 µl	- 20 °C
Deionized H_20	Deionized H_20	2 x 1 ml	2 x 1 ml	- 20 °C
Genomic DNA or recombinant DNA	Positive control 1 Ins 5bp CALR Del 52bp CALR	1 x 40 µl	1 x 40 µl	- 20 °C
Genomic DNA or recombinant DNA	Negative control Housekeeping	1 x 40 µl	1 x 40 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, tissue, cells
POSITIVE CONTROL	Recombinant DNA for at least 3 analytical sessions
NEGATIVE CONTROL	Recombinant DNA for at least 3 analytical sessions
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	85 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
ANALYTICAL SENSITIVITY : LIMIT OF DETECTION (LOD)	≥ 0,025 ng of DNA, < 1%
ANALYTICAL SENSITIVITY : LIMIT OF BLANK (LOB)	0% NCN
REPRODUCIBILITY	99,9%
DIAGNOSTIC SPECIFICITY / DIAGNOSTIC SENSITIVITY	100%/98%

BIOMOL LABORATORIES S.R.L.
Via Arcora 110 (Palazzo Gecos)
80013 Casalnuovo di Napoli, NA
info@biomollaboratories.com
biomollaboratories.it

ISO 9001:2015
ISO 13485:2016

BioMol LABORATORIES