# GENETIC VARIANTS OF THE ENZYME CYTOCHROME P450 CYP2C9 (variants *2 and *3)

For in vitro diagnostic use

CE IVD

## ORDERING INFORMATIONS

*REF: FGC-005-25*
*RDM Code: 1973964/R*
*CND Code: W0106030101*
*Tests: 25*
*Reactions: 31 x 2*
*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

Device belonging to the family of in vitro medical devices **REAL-TIME QUALITATIVE PCR-GENETIC VARIANTS.** Detection of the genetic variants rs1799853 (*2) and rs1057910 (*3) of the CYP2C9 gene by amplification with oligonucleotides and specific probes (allele-specific genotyping) and subsequent detection with qPCR-Real-time. Kit optimized for Real-Time PCR instrumentation Biorad CFX96 Dx, Biorad Opus Dx and Agilent AriaDx.

## SCIENTIFIC BACKGROUND

Cytochromes P450s are a family of enzymes responsible for approximately 75% of all drug metabolism reactions. There are multiple isoforms of cytochrome P450 but most reactions are metabolised by CYP2C9, CYP2C19, CYP2D6 and CYP3A4. The CYP2C9 gene has been mapped to chromosome 10q24.2 and is highly variable; nucleotide sequencing has already identified nearly 60 alleles. Three alleles, namely CYP2C9 *1 (the wild-type allele with normal activity), CYP2C9 *2 and CYP2C9 *3 (both with reduced enzyme activity) are often identified in studies in the Caucasian population. Among the 60 variant CYP2C9 star (*) alleles listed on The Pharmacogene Variation Consortium website (https://www.pharmvar.org) at least 20 are reported to have in vivo and/or in vitro functional evidence of altered activity.

The CYP2C9 * 2 variant (rs1799853) has a C>T transition at position 430 of exon 3 coding for arginine, resulting in a substitution at position 144 (Arg144Cys) of the CYP2C9 protein, while the analysis of the CYP2C9 *3 variant (rs1057910) demonstrated an A>C transversion at position 1075 in exon 7 causing an isoleucine to leucine substitution at position 359 (Ile359Leu).


§ Mol Biol Rep. 2024 Jan 16;51(1):105.Genetic variation of CYP2C9 gene and its correlation with cardiovascular disease risk factors
§ Nucleosides Nucleotides Nucleic Acids. 2024;43(4):356-376. Identification of the effects of pathogenic genetic variations of human CYP2C9 and CYP2D6: an in silico approach.
§ Recommendations for Clinical CYP2C9 Genotyping Allele Selection: A Joint Recommendation of the Association for Molecular Pathology and College of American Pathologists.
The Journal of molecular diagnostics. JMD. 2019.
§ Polymorphisms of CYP2C9*2, CYP2C9*3 and VKORC1 genes related to time in therapeutic range in patients with atrial fibrillation using warfarin. Appl Clin Genet. 2019 Aug 2;12:1 51-159.
§ The Cytochrome P450 Slow Metabolizers CYP2C9*2 and CYP2C9*3 Directly Regulate Tumorigenesis via Reduced Epoxyeicosatrienoic Acid Production. Cancer Res. 2018 Sep 1; 78 (17):4865-4877.
§ CYP2C9 polymorphisms in epilepsy: influence on phenytoin treatment. Pharmgenomics Pers Med. 2018 Mar 29;11: 51-58.
§ Applications of CYP450 testing in the clinical setting. Mol Diagn Ther. 2013 Jun; 17(3):165-84.


## CLINICAL SIGNIFICANCE

The cytochrome P450 superfamily is mainly expressed in the liver, small intestine and kidney. CYP P450 enzymes catalyze several types of oxidation and some reduction reactions.

Genetic polymorphisms in CYP genes are the major cause of inter-individual variation in drug metabolism. They cause variations in drug response ranging from adverse effects to lack of efficacy. In addition, CYP polymorphisms have been reported to confer susceptibility or reduced risk/protection from disease. CYP2C9 plays an important role in the phase I metabolism of xenobiotics and some endogenous compounds, for example, nonsteroidal anti-inflammatories, oral anticoagulants and oral hypoglycaemics. Individuals with low CYP2C9 catalytic activity (poor and/or intermediate metabolisers) develop adverse drug reactions particularly with substrates with a narrow therapeutic index, e.g. S-warfarin, phenytoin, glipizide and tolbutamide. The combination of genotypes (www.pharmgkb.org) allows to define the patient as "Normal Metabolizer" (Homozygous CYP2C9*1), "Intermediate Metabolizer" (Heterozygous CYP2C9*2 and *3) and "Poor Metabolizer" (Homozygous or Double CYP2C9 heterozygous *2 and *3).

Recently, the importance of CYP2C9 in the metabolism of Siponimod, an orally available immunomodulatory drug used to treat relapsing forms of multiple sclerosis, has been demonstrated. Indeed, the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) indicate that molecular characterization is necessary before starting treatment (CYP2C9 *3/*3 patients should not be subjected to pharmacological treatment).


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



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ISO 13485:2021

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| DESCRIPTION | LABEL | VOLUME | STORAGE |
|---|---|---|---|
| | | FGC-005-25 | |
| Mix oligonucleotides and probes | Mix 10X CYP2C9 *2 | 1 x 77,5 µl | -20°C |
| Mix oligonucleotides and probes | Mix 10X CYP2C9 *3 | 1 x 77,5 µl | -20°C |
| Mix buffer and Taq-polymerase enzyme | Mix Real-Time PCR 2X | 1 x 775 µl | -20°C |
| Deionized $H_20$ | Deionized $H_20$ | 1 x 1 ml | -20°C |
| Genomic DNA or recombinant DNA Control 1 | Control 1<br>Homozygous **CC CYP2C9 C430T**<br>Homozygous **AA CYP2C9 A1075C** | 1 x 30 µl | -20°C |
| Genomic DNA or recombinant DNA Control 2 | Control 2<br>Heterozygous **CT CYP2C9 C430T *2**<br>Heterozygous **AC CYP2C9 A1075C *3** | 1 x 30 µl | -20°C |

## TECHNICAL CHARACTERISTICS

COD. FGC-005-25

| | |
|---|---|
| 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 |
| TECHNOLOGY | Real-time PCR; oligonucleotides and specific probes; 2 FAM/HEX fluorescence channels |
| VALIDATED INSTRUMENTS | Biorad CFX96 Dx, Biorad Opus Dx e Agilent AriaDx |
| RUNNING TIME | 85 min |
| THERMAL CYCLING PROFILE | 1 cycle at 95 °C (10 min); 45 cycles at 95 °C (15 sec) + 60 °C (60 sec) |
| ANALYTICAL SPECIFICITY | Absence of non-specific pairings of oligonucleotides and probes; absence of cross-reactivity |
| LIMIT OF DETECTION (LOD) | ≥ 0,016 ng of genomic DNA |
| LIMIT OF BLANK (LOB) | 0% NCN |
| REPRODUCIBILITY | 99,9% |
| DIAGNOSTIC SPECIFICITY / DIAGNOSTIC SENSITIVITY | 100%/98% |

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