KRAS CE IVD

TECHNICAL SHEET IDYLLA™ KRAS MUTATION TEST

The **Idylla™ KRAS Mutation Test,** performed on the Biocartis Idylla™ system, is an *in vitro* diagnostic Test for the qualitative detection of 21 mutations in **codons 12, 13, 59, 61, 117 and 146** of the *KRAS* gene. The Idylla™ KRAS Mutation Test, from **sample-to-result**, starts with formalin-fixed paraffin-embedded (FFPE) human tissue from metastatic colorectal cancers to liberate DNA for subsequent real-time PCR amplification and detection.

FEATURES

KRAS mutation detection		
Codon 12 (exon 2)	G12C	(c.34G>T)
	G12R	(c.34G>C)
	G12S	(c.34G>A)
	G12A	(c.35G>C)
	G12D	(c.35G>A)
	G12V	(c.35G>T)
Codon 13 (exon 2)	G13D	(c.38G>A)
Codon 59 (exon 3)	A59E	(c.176C>A)
	A59G	(c.176C>G)
	A59T	(c.175G>A)
Codon 61 (exon 3)	Q61K	(c.181C>A; c.180_181delinsAA)
	Q61L	(c.182A>T)
	Q61R	(c.182A>G)
	Q61H	(c.183A>C; c.183A>T)
Codon 117 (exon 4)	K117N	(c.351A>C; c.351A>T)
Codon 146 (exon 4)	A146P	(c.436G>C)
	A146T	(c.436G>A)
	A146V	(c.437C>T)
KRAS Total (acting as Sample Processing Control)		

Specimen requirements	
Sample Type	FFPE tissue sections (5 to 10 µm)
Neoplastic cells	≥10%, if less macrodissection is required
Tissue area	50-600 mm^2 (5 µm) 25-300 mm^2 (10 µm)

Performance	
Analytical Sensitivity	LOD ≤5% for all KRAS mutations

Between Laboratory Reproducibility (480 results at 3 sites)	100% agreement for 5% KRAS G12D 100% agreement for 5% KRAS G12S 100% agreement for 5% KRAS G12V 100% agreement for 50% KRAS G13D
Between Lot Reproducibility (375 results on 3 lots)	100% agreement for 5% KRAS G12A 100% agreement for 5% KRAS G12D 100% agreement for 5% KRAS G12S 100% agreement for 5% KRAS G12V 100% agreement for 5% KRAS G13D
Total turnaround time	
Time	120 minutes

ACCURACY – CLINICAL PERFORMANCE EVALUATION

96.7% overall percent agreement was obtained during the clinical performance evaluation comparing Idylla™ with a reference method based on RT-PCR.

96.7% overall concordance		Reference test								
		G12A	G12C	G12D	G12R	G12S	G12V	G13D	No mutation	Total
Idylla™ KRAS Mutation Test	G12A	**6**								6
	G12C		**6**							6
	G12D			**25**						25
	G12R				**3**				**1***	4
	G12S					**6**				6
	G12V					**1**	**15**		**1***	17
	G13D					**1**		**16**	**3***	20
	No mutation	**1***							**97**	98
	A59E/G/T								1	1
	Q61H/H2								3	3
	Q61K/K2									0
	Q61L/R									0
	K117N/N2		1						2	3
	A146T/V/P						1		4	5
	Totals	7	7	25	3	8	16	16	112	194

Note: the Reference test is not designed to pick up mutations in codon 59, 61, 117 and 146

* Due to limitations in available material (n=2) or insufficient DNA quality (n=3), only one of the 6 discordant results could be resolved; NGS confirmed the G12V result called by Idylla™

Discordant analysis by NGS

100% overall concordance | Reference test and further analysis by NGS

		G12A	G12C	G12D	G12R	G12S	G12V	G13D	No mutation	Total
Idylla™ KRAS Mutation Test	G12A	**6**								6
	G12C		**6**							6
	G12D			**25**						25
	G12R				**3**					3
	G12S					**6**				6
	G12V					**1**	**16**			17
	G13D					**1**		**16**		17
	No mutation								**97**	97
	A59E/G/T								1	1
	Q61H/H2								3	3
	Q61K/K2									0
	Q61L/R									0
	K117N/N2								2	3
	A146T/V/P		1				1		4	5
	Totals	6	7	25	3	8	17	16	107	189

MULTI-CENTER EVALUATION OF THE FULLY-AUTOMATED PCR-BASED IDYLLA™ KRAS MUTATION ASSAY FOR RAPID KRAS MUTATION STATUS DETERMINATION ON FORMALIN-FIXED PARAFFIN-EMBEDDED TISSUE OF HUMAN COLORECTAL CANCER.

Solassol J. et al. PLOS ONE 2016.

95.9% overall concordance | Routine reference methods*

		Codon 12	Codon 13	Codon 59	Codon 61	Codon 117	Codon 146	No mutation	Total
Idylla™ KRAS Mutation Assay	Codon 12	**138**						**3**	141
	Codon 13		**38**						38
	Codon 59			**5**					5
	Codon 61	1			**14**			**5**	20
	Codon 117					**4**			4
	Codon 146						**18**	**1**	19
	No mutation	**3**	**2**				**1**	**129**	135
	TOTAL	142	40	5	14	4	19	138	362

Mutations not picked up by Idylla™ or the reference method (n=8) are not included in the % agreement calculation. Idylla does not pick up the following low prevalent (<1%) mutations: 4xG13C, 1xG13R, 1xG12F (samples excluded from table). Roche Cobas does not pick up mutations in codon 146 (2x).

* Different reference methods were used: cobas® KRAS Mutation Test (Roche), Ion Torrent AmpliSeq™ Colon and Lung Cancer Research Panel (Life Technologies), therascreen® KRAS Pyro® Kit (Qiagen), therascreen® RAS Extension Pyro Kit (Qiagen), HRM screening and pyrosequencing, Sanger sequencing, HRM screening and Sanger sequencing; for the analysis, when Idylla™ identified a specific mutation in codon 12, 13 or 61, and the cobas® KRAS Mutation Test (Roche) reported a "codon 12/13" or "codon 61" result), both results were considered identical.

↓ Discordant analysis by ddPCR, Idylla™ retest and NGS

98.9% overall concordance		Routine reference methods, including further analysis by ddPCR, Idylla™ retest, NGS							
		Codon 12	Codon 13	Codon 59	Codon 61	Codon 117	Codon 146	No mutation	Total
Idylla™ KRAS Mutation Assay	Codon 12	**142**							142
	Codon 13		**38**						38
	Codon 59			**5**					5
	Codon 61	**1**			**15**			**1**	17
	Codon 117					**4**			4
	Codon 146						**20**		20
	No mutation	**1**	**2**					**133**	136
	TOTAL	144	40	5	15	4	20	134	362

IDYLLA™ KRAS POSTERS & PUBLICATIONS

- Maertens G. et al. A solution for same-day extended RAS testing. Poster ESMO 2015
- Vandenbroucke I. et al. A rapid and fully automated multiplex assay for KRAS-BRAF mutations with high mutation sensitivity using novel selective amplification and detection technologies. Poster AACR 2014
- Solassol J. et al. Multi-Center Evaluation of the Fully Automated PCR-Based Idylla™ KRAS Mutation Assay for Rapid KRAS Mutation Status Determination on Formalin-Fixed Paraffin-Embedded Tissue of Human Colorectal Cancer. PLOS ONE 2016
- Weyn C. et al. Clinical performance evaluation of a sensitive, rapid low-throughput test for KRAS mutation analysis using formalin-fixed, paraffin-embedded tissue samples. BMC Cancer 2017

RESEARCH APPLICATIONS

- Dario de Biase. et al. Fully automated PCR detection of KRAS mutations on pancreatic endoscopic ultrasound fine-needle aspirates. J Clin Path 2016.

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