Details on In-House Molecular Testing

Biocartis Idylla™ System

The Biocartis Idylla™ System is a fully automated sample-to-result PCR based molecular diagnostic system. It covers the entire process from sample to result with fully integrated sample preparation followed by real-time PCR amplification and detection of the targeted sequences.

The Idylla™ System consists of:

- The Idylla™ Console connected to one or more Idylla™ Instruments
- Idylla™ Cartridges designed for a specific application

The test cartridges are ready-for-use and contain the necessary reagents to perform sample preparation and real-time PCR amplification and detection. The process steps in the test are:

- Formalin-fixed paraffin-embedded (FFPE) tissue liquefaction and cell lysis: After insertion of the FFPE tissue section into the cartridge, a combination of chemical reagents, enzymes, heat, and High Intensity Focused Ultrasound (HIFU) induces deparaffinisation, disruption of the tissue and lysis of the cells. The nucleic acids are liberated for subsequent PCR amplification.
- Real-time PCR using allele-specific primers: The DNA is amplified, and target sequences are detected by means of fluorescence. PCR reagents are present in a stable formulation in the PCR chambers. Each chamber contains its specific reagents related to the target to detect.
- At the end of the run, a final report can be obtained on the console screen indicating the presence or absence of specific mutations or the invalidity of the test run.

If no mutation is detected, the presence of a mutation might not be excluded since the result is dependent on:

- the integrity of the specimen DNA
- the percentage of mutant sequences present in the specimen
- the absence of inhibiting substances
- the presence of sufficient amplifiable DNA

Details on Specific Tests:

BRAF for melanoma:

The Idylla™ BRAF Mutation Assay detects V600E/E2/D and V600K/R/M mutations in codon 600 of the BRAF gene. It can detect these mutations at an analytical sensitivity of 1% of mutant in a wild type genomic DNA background in FFPE samples. These mutations account for >90% of BRAF Codon 600 mutations known to be clinically significant. The percentage of neoplastic cells required for this assay is >50%. Mutations outside the codons analysed cannot be detected. Due to its specific primer and probe design, V600E, V600E2 and V600D mutations cannot be distinguished from one another. The same applies for the V600K, V600R and V600M mutations.

The assay consists of three allele-specific duplex PCR reactions, designed to specifically amplify the BRAF Wild Type, V600E, V600E2, V600D, V600K, V600R and V600M mutations, each combined with an endogenous control gene that serves as a Sample Processing Control (SPC). This control checks for adequate execution of the complete process from sample to result.

List of mutations detected by the Idylla™ BRAF Mutation Assay for Melanoma

Exon	Codon	Mutation	Amino Acid Change	Nucleotide Change	Genetic Call Idylla™ BRAF
15	600	V600E	p.Val600Glu	c.1799T>A	V600E/E2/D
		V600E2	p.Val600Glu	c.1799_1800TG>AA	
		V600D	p.Val600Asp	c.1799_1800TG>AT c.1799_1800TG>AC	
		V600K	p.Val600Lys	c.1798_1799GT>AA	V600K/R/M
		V600R	p.Val600Arg	c.1798_1799GT>AG	
		V600M	p.Val600Met	c.1798G>A	

KRAS for colorectal carcinoma:

The Idylla™ KRAS Mutation Test is an in vitro diagnostic test for the qualitative detection of mutations in codons 12, 13, 59, 61, 117 and 146 of the KRAS oncogene in DNA derived from FFPE human colorectal cancer (CRC) tissue. Mutations outside the codons analysed cannot be detected. This assay is designed to detect 95% of all KRAS mutations known to be clinically significant and has an analytical sensitivity of 5% for all KRAS mutations. The presence of >10% neoplastic cells from one tissue section is recommended. All results should be interpreted in conjunction with the clinical details.

The test consists of five allele-specific multiplex PCR reactions, designed to specifically amplify KRAS gene sequences with a mutation in codons 12, 13, 59, 61, 117 and 146. A conserved fragment in the intron 4/exon 5 junctional region of the KRAS gene is amplified simultaneously. This serves as a sample processing control (SPC) that checks for adequate execution of the complete process from sample to result, and is present in each of the five multiplexes. In addition, this control reaction is a measure for the amount of amplifiable DNA in the sample and is used in the analysis of the mutation status of the sample.

List of KRAS mutations detected by the Idylla™ KRAS Mutation Test

Exon	Codon	Mutation	Amino Acid Change	Coding DNA Change	Genetic Call Idylla™ KRAS
2	12	G12A	p.Gly12Ala	c.35G>C	G12A
		G12C	p.Gly12Cys	c.34G>T	G12C
		G12D	p.Gly12Asp	c.35G>A	G12D
		G12R	p.Gly12Arg	c.35G>C	G12R
		G12S	p.Gly12Ser	c.34G>A	G12S
		G12V	p.Gly12Val	c.35G>T	G12V
	13	G13D	p.Gly13Asp	c.38G>A	G13D
3	59	A59E	p.Ala59Glu	c.176C>A	A59T/E/G
		A59G	p.Ala59Gly	c.176C>G	
		A59T	p.Ala59Thr	c.175G>A	
	61	Q61H	p.Gln61His	c.183A>C	Q61H
		Q61H	p.Gln61His	c.183A>T	
		Q61K	p.Gln61Lys	c.181C>A	Q61K
		Q61K	p.Gln61Lys	c.180_181delinsAA	
		Q61L	p.Gln61Leu	c.182A>T	Q61L/R
		Q61R	p.Gln61Arg	c.182A>G	
4	117	K117N	p.Lys117Asn	c.351A>C	K117N
		K117N	p.Lys117Asn	c.351A>T	
	146	A146P	p.Ala146Pro	c.436G>C	A146P/T/V
		A146T	p.Ala146Thr	c.436G>A	
		A146V	p.Ala146Val	c.437C>T	

NRAS and BRAF for colorectal carcinoma:

The Idylla™ NRAS-BRAF Mutation Test is an in vitro diagnostic test for the qualitative detection of mutations in codons 12, 13, 59, 61, 117 and 146 of the NRAS gene and in codon 600 of the BRAF gene in DNA derived from FFPE tissue. Mutations outside the codons analysed cannot be detected. This assay is designed to detect 95% of all NRAS-BRAF mutations known to be clinically significant and has an analytical sensitivity of 5% for the most prevalent NRAS-BRAF mutations. The presence of >10% neoplastic cells from one tissue section is recommended. All results should be interpreted in conjunction with the clinical details.

The test consists of five allele-specific multiplex PCR reactions, designed to specifically amplify NRAS and BRAF gene sequences with a mutation. Conserved regions of the NRAS gene and BRAF gene are amplified simultaneously. These PCR reactions serve as sample processing controls (SPC) that check for adequate execution of the complete process from sample to result. In addition, these control reactions are a measure for the amount of amplifiable DNA in the sample and are used in the analysis of the mutation status of the sample.

List of NRAS mutations detected by the Idylla™ NRAS-BRAF Mutation Test

Exon	Codon	Mutation	Amino Acid Change	Nucleotide Change	Genetic Call Idylla™ NRAS
2	12	G12A	p.Gly12Ala	c.35G>C	G12A/V
		G12V	p.Gly12Val	c.35G>T	
		G12C	p.Gly12Cys	c.34G>T	G12C
		G12D	p.Gly12Asp	c.35G>A	G12D
		G12S	p.Gly12Ser	c.34G>A	G12S
	13	G13D	p.Gly13Asp	c.38G>A	G13D
		G13R	p.Gly13Arg	c.37G>C	G13R/V
		G13V	p.Gly13Val	c.38G>T	
3	59	A59T	p.Ala59Thr	c.175G>A	A59T
	61	Q61H	p.Gln61His	c.183A>C	Q61H
		Q61H	p.Gln61His	c.183A>T	
		Q61K	p.Gln61Lys	c.181C>A	Q61K
		Q61L	p.Gln61Leu	c.182A>T	Q61L
		Q61R	p.Gln61Arg	c.182A>G	Q61R
4	117	K117N	p.Lys117Asn	c.351G>C	K117N
		K117N	p.Lys117Asn	c.351G>T	
		A146T	p.Ala146Thr	c.436G>A	A146T/V
		A146V	p.Ala146Val	c.437C>T	

List of BRAF mutations detected by the Idylla™ NRAS-BRAF Mutation Test

Exon	Codon	Mutation	Amino Acid Change	Nucleotide Change	Genetic Call Idylla™ KRAS
15	600	V600E	p.Val600Glu	c.1799T>A	V600E/D
				c.1799_1800delinsAA	
		V600D	p.Val600Asp	c.1799_1800delinsAC	
		V600K	p.Val600Lys	c.1798_1799delinsAA	V600K/R
		V600R	p.Val600Arg	c.1798_1799delinsAG	