Modaplex

POLE/POLD1 MUTATION TESTING

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BIOTYPE

POLE AND POLD1 IN CLINICAL RESEARCH

PROMISING CANDIDATES FOR CLINICAL PRACTICE

POLE AND POLD1 AS RESEARCH MARKER FOR IMMUNE-CHECKPOINT INHIBITION

Mutations in the exonuclease domain (EDMs) of the catalytic subunits of the DNA polymerases epsilon and delta 1 (POLE & POLD1) lead to impaired proofreading during DNA replication, thereby dramatically increasing the mutation rates[1].

Clinical researchers are now investigating how POLE/POLD1 exonuclease mutations could complement MSI testing to facilitate decision making for immune-checkpoint therapies[2,3,4].

INVESTIGATION OF POLE MUTATIONS IN ENDOMETRIAL CARCINOMA

The Cancer Genome Atlas Research Network (TCGA) performed an integrating genomic, transcriptomic, and proteomic characterization of endometrial carcinoma. Exome sequence analysis revealed four groups of tumors[5].

- **Group 1** carcinomas have somatic inactivating hotspot mutations in the POLE exonuclease domain and a very high mutational burden (ultramutated).
- **Group 2** includes endometrioid carcinomas which are microsatellite instable (MSI) (hypermutated), and present frequently with MLH-1 promoter hypermethylation and high mutation rates.
- **Group 3** tumors often have low copy number alterations, and a low mutational burden, while lacking POLE mutations and MSI-H.
- **Group 4** (Serous-like or copy-number high) show a low mutation rate, nearly universal TP53 mutations, and have a highly unfavorable prognosis.

Clinical researchers are now attempting to bring the TCGA molecular-based classification into clinical practice[6].

REFERENCES

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2 R. Bourdais et al, "Polymerase proofreading domain mutations: New opportunities for immunotherapy in hypermutated colorectal cancer beyond MMR deficiency", Crit. Rev. Oncol. Hematol., vol. 113, pp. 242-248, 2017.
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INTRODUCING THE MODAPLEX POLE/POLD1 SOLUTION

INVESTIGATE POLE/POLD1 EXONUCLEASE DOMAIN MUTATIONS WITH MODAPLEX POLE/POLD1 MUTATION ANALYSIS KIT

GET ACCESS TO REPRODUCIBLE POLE/POLD1 TEST PERFORMANCE

The Modaplex POLE/POLD1 Kit is a fully commercially developed and documented, PCR-based multiplex assay. In this context, users can rely on a substantial amount of analytical performance data that ensures performance reproducibility and reliability. Furthermore, the data represent the basis for a further development to an IVD test.

OBTAIN MEANINGFUL RESULTS ANALYSING A SET OF POLE/POLD1 EDMS

The Modaplex POLE/POLD1 Mutation Analysis Kit enables users to detect and differentiate sixteen (16) POLE and three (3) POLD1 exonuclease domain mutations.

BENEFIT FROM AN EFFICIENT WORKFLOW

The POLE/POLD1 assay is designed for use with the Modaplex instrument, a powerful multiplex PCR bench-top system serving you with a load- and walk-away workflow. As the workflow is identical for all tests and combination of tests, it allows clinical researchers to set-up POLE/POLD1 mutation testing simultaneously with other tests such as MSI fragment analysis.

MODAPLEX POLE/POLD1 MUTATION ANALYSIS KIT

OBTAIN MEANINGFUL RESULTS WITH THIS SET OF POLE/POLD1 MUTATIONS

Detection and differentiation of nineteen (19) somatic and rare germline mutations in the polymerase epsilon/polymerase delta-1 exonuclease domains is effectively achievable using the Modaplex POLE/POLD1 Mutation Analysis Kit. The test enables researchers to advance promptly in clinical research, as the test is optimized to evaluate POLE and POLD1 mutations that have been described to be:

- predominant in colorectal and endometrial cancer, but have also been reported for gastric, breast and brain cancers[1,2,3,4,10]
- primarily associated with high mutational burden showing a very distinct mutational pattern[5,6,11]
- associated with high expression of immune-checkpoint proteins and T-cell markers[5,6,9]
- predominantly present in cancer samples with a MSS-phenotype[4,7,8]

MODAPLEX POLE/POLD1 TARGET LIST

Single Tube qualitative 21 plex reaction

POLE	T278M	S297F	P286L	P286H
	S297A	M444K	V411L (G>C)	V411L (G>T)
	P436R	F367S	A456P	S459F
	P286R	L424V	A465V	
	H422N			
POLD1	D316N	C319Y	S478N	
Internal Controls	IC1	IC2		

REFERENCES

1 E. Rayner et al., A panoply of errors: polymerase proofreading domain mutations in cancer. Nat Rev Cancer 16, 71–81 (2016).
2 R. Bourdais et al., Polymerase proofreading domain mutations: New opportunities for immunotherapy in hypermutated colorectal cancer beyond MMR deficiency. Crit Rev Oncol Hematol. 2017; vol. 113: 242-248.
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5 J.M. Mehnert et al, Immune activation and response to pembrolizumab in POLE-mutant endometrial cancer, J. Clin. Invest., 2016;126(6):2334-40
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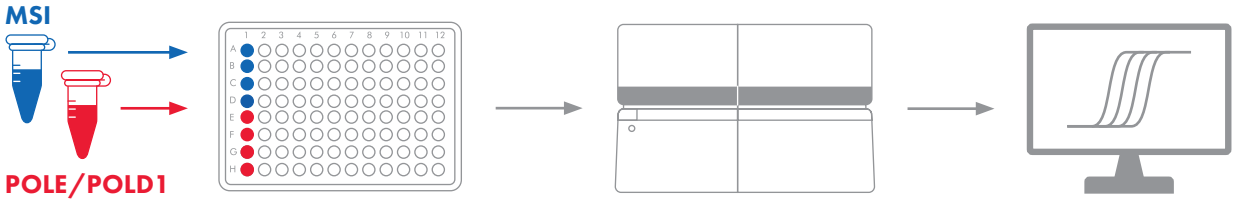
MODAPLEX POLE/POLD1 WORKFLOW

BENEFIT FROM AN EFFICIENT WORKFLOW

It has become apparent that the complete evaluation of genetic conditions requires the simultaneous analysis of a multitude of molecular markers. Using multi-gene sequencing panels will provide insight into many genomic alterations: But they are often too large and produce unused data through a complex and time-consuming workflow.

Because Modaplex assays make use of a universal PCR program, you can perform these assays simultaneously using a workflow which is as simple as setting up a PCR. Thus POLE/POLD1 mutation detection and microsatellite fragment analysis can be easily combined and flexibly performed in a single Modaplex run in less than 3,5 hours.



PCR set up
15 min

Automated Modaplex run
~ 3 hrs

Analysis with Moda-RA
15 min

3.5 hrs

ADDITIONAL MODAPLEX POLE/POLD1 FEATURES

Scalable in use. Investigate up to 46 samples (incl. controls)

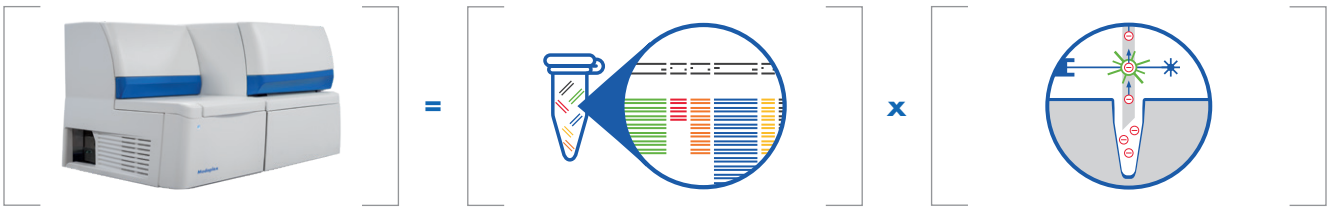
Optimized to 4ng sample input to save tissue

Endowed with a comprehensive control concept to create reliability

Equipped with all PCR reagents

MODAPLEX TECHNOLOGY

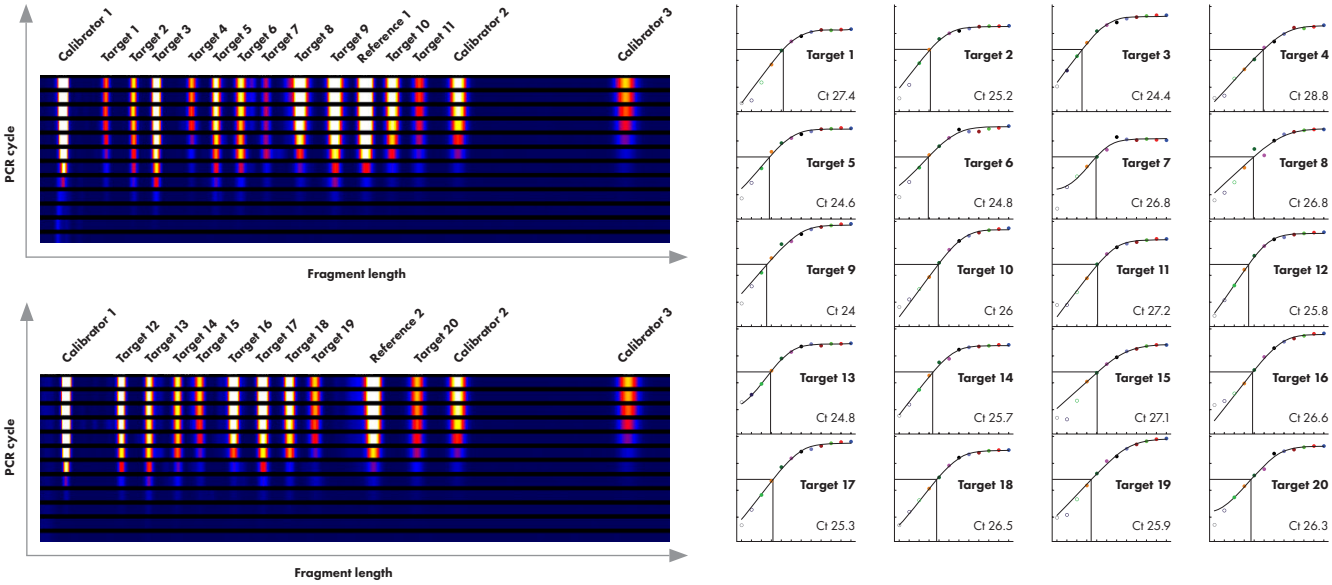
UNIQUE ABILITIES, PROVEN TECHNOLOGIES



MODAPLEX | **PCR** | **CAPILLARY GEL ELECTROPHORESIS**

Quantitative PCR (qPCR) is cost-effective, fast, and sensitive, but it is limited to the number of fluorophores that can be combined in one qPCR run. In the Modaplex system this limitation is overcome through the use of size separation. A Modaplex PCR reaction is run with fluorescently-labelled primers. During PCR, these amplicons are injected electro-kinetically into the capillary gel. While the PCR reaction continues undisturbed, the injected PCR products migrate through the gel in a size-dependent manner. By combining the size separation and detection after the PCR cycle, real-time data is generated.

UNIQUE REAL-TIME QUANTIFICATION PROCEDURE



During PCR, labelled amplicons are separated by size and their individual fluorescence is measured. Since multiple measurements are made during the PCR, an amplification curve is formed, from which the threshold cycle (Ct) for each amplicon is determined.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ORDER INFORMATION

Product	Cat. no.	Application
Modaplex POLE/POLD1 Mutation Analysis Kit	85-10101-0050	RUO
Modaplex Instrument	00-04901-0001	

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