Package-Ref ™ MRD Cocktail Reference Standard

CBP90040

BACKGROUND

Minimal residual disease or Measurable Residual Disease or Molecular Residual Disease (MRD) refers to the small number of cancer cells that remain in the body after treatment. The most widely used tests are flow cytometry, polymerase chain reaction (PCR) and next-generation sequencing (NGS).

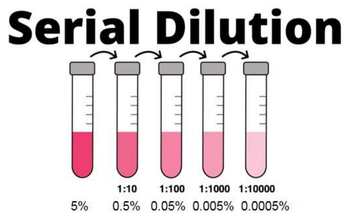
There are two main NGS technical routes for MRD detection of ctDNA: ***tumor-agnostic -assays*** and ***tumor-informed assays***. Tumor-agnostic -assays only detect plasma, using fixed panels (usually driver genes and targeted drug genes, and multi-omics methods) and analytical methods to detect and analyze ctDNA. Tumor-informed assays require sequencing of primary tumor tissue (usually WES) to identify the patient's specific genomic variation map, and then customize a personalized panel to test ctDNA, which means that both tissue and plasma need to be tested.

INTRODUCTION

For ctDNA MRD diagnostic performance evaluation, cb-gene has developed an ultra-low frequency ctDNA standard by Ultrasonic interruption，It covers a wide range of genes and sites and has rich mutation types.

Catalog	Name	Quantity	Note
CBP90040-1	Package-RefTM MRD Cocktail Reference Standard-0.5%	500 ng/vial	Optimized ddPCR assay
CBP90040-2	Package-RefTM MRD Cocktail Reference Standard-0.05%	500 ng/vial	Dilution fold-confirmation
CBP90040-3	Package-RefTM MRD Cocktail Reference Standard-0.005%	500 ng/vial	Dilution fold-confirmation
CBP90040-4	Package-RefTM MRD Cocktail Reference Standard-0%	500 ng/vial	ddPCR assay

PRODUCT DATA



How to verify %AF which is ultra-low?

CB-gene adopts methods 1 and 4.

1. Improve the performance of ddPCR assay;
2. ultra-Deep sequencing of NGS;
3. To check the dilution-fold with the non-human sequence;
4. To check the dilution-fold with the high-%AF mutations.

	GENE	Mut.	CDS change	Variant Classification	AF%
MRD-0.5% ctDNA	EGFR	p.E746_A750delELREA	c.2236_2250del15GAATTAAGAGAAGCA>-	Pathogenic	0.69
	EGFR	p.S768I	c.2303G>T	Pathogenic	0.61
	EGFR	p.L861Q	c.2582T>A	Pathogenic	0.61
	ERBB2	p.A775_G776insYVMA	c.2324_2325ins12ATACGTGATGGC	Pathogenic	0.41
	KRAS	p.G12V	c.35G>T	Pathogenic	0.39
	PIK3CA	p.E542K	c.1624G>A	Pathogenic	0.62
	KRAS	p.Q61H	c.183A>C	Pathogenic	0.49
	EGFR	p.G719C	c.2155G>T	Pathogenic	0.5
	ROS1	CD74-ROS1	chr5:149783684:- (hg19) chr6:117646732:- (hg19)	Pathogenic	0.55
	NRAS	p.Q61K	c.181C>A	Pathogenic	0.41
	IDH1	p.R132C	c.394C>T	Pathogenic	0.43
	FBXW7	p.R505C	c.1513C>T	Pathogenic	0.75
	KMT2C	p.F4496Lfs*21	c.13488delA	Likely Pathogenic	0.46
	RAC1	p.N92I	c.275A>T	Pathogenic	0.4
	BCOR	p.Q1321*	c.3961C>T	Likely Pathogenic	0.68
	BRCA2	p.M784V	c.2350A>G	Benign	0.64
	ARID1A	p.G1848Wfs*6	c.5540dupG	Likely Pathogenic	0.61
	CDH1	p.P126Rfs*89	c.377delC	Pathogenic	0.74
	BARD1	p.K208Rfs*4	c.623delT	Likely Pathogenic	0.38
	BAX	p.E41Rfs*19	c.121delG	Pathogenic	0.75
	NBN	p.R466Gfs*18	c.1396delT	Pathogenic	0.71
	KMT2D	p.Q809Rfs*121	c.2425delG	Likely Pathogenic	0.51
	KMT2D	p.P647Hfs*283	c.1940delG	Pathogenic	0.44
	QKI	p.K134Rfs*14	c.401delA	Likely Pathogenic	0.63
	KMT2B	p.W336*	c.1007G>A	Likely Pathogenic	0.75
	NSD1	p.M1531Cfs*43	c.4591delA	Likely Pathogenic	0.74
	AXIN1	p.E31*	c.91G>T	Likely Pathogenic	0.66
	AXIN1	p.G508Vfs*197	c.1523del	Likely Pathogenic	0.69
	ARID1B	p.A547Sfs*35	c.1638_1647delGGGCGGCGGC	Pathogenic	0.47
	HLA-C	p.V100Efs*38	c.299_300delTG	Likely Pathogenic	0.51
	TP53BP1	p.R1447Efs*36	c.4339delC	Likely Pathogenic	0.57
	POLD1	N/A	c.E24-2AG>A	Likely Pathogenic	0.73
	CYLD	p.N722Mfs*13	c.2165delA	Likely Pathogenic	0.57
	INHA	p.G66Afs*61	c.197delG	Likely Pathogenic	0.74
	RASA1	p.N838Mfs*4	c.2513delA	Likely Pathogenic	0.51
	RECQL4	p.G952Afs*92	c.2855delG	Likely Pathogenic	0.54
	BRAF	p.V600E	c.1799T>A	Pathogenic	5.33
	ACVR2A	p.K437Rfs*5	c.1310delA	Likely Pathogenic	3.98
	PIK3CA	p.H1047R	c.3140A>G	Pathogenic	2.81
	BRCA1	p.D435Y	c.1303G>T	Uncertain significance	2.53
	BRCA2	p.N1784Tfs*7	c.5351delA	Pathogenic	2.35
	BRCA2	p.E2292A	c.6875A>C	Uncertain significance	1.35
	TP53	N/A	c.783-2A>C	Pathogenic	1.02
	CDKN2A	p.R80*	c.238C>T	Pathogenic	0.92
	HLA-A	p.R45Afs*32	c.133del	Likely Pathogenic	1.25

	GENE	Mutation	AF% of MRD-0.5% ctDNA	AF% of MRD-0.05% ctDNA	AF% of MRD-0.005% ctDNA
Dilution flod verification	BRAF	p.V600E	5.33	0.54	0.047
	PIK3CA	p.H1047R	2.81	0.26	N/A
	BRCA1	p.D435Y	2.53	0.24	N/A
	BRCA2	p.N1784Tfs*7	2.35	0.23	N/A
	TP53	c.783-2A>C	1.02	0.11	N/A
	CDKN2A	p.R80*	0.92	0.1	N/A
	HLA-A	p.R45Afs*32	1.25	0.11	N/A

The ddPCR detection system accurately calibrated the AF%.

- selected 8 genes and 9 loci as the dilution fold verification of 0.05%
- selected 2 genes and 2 loci as the dilution fold verification of 0.005%.

ACVR2A	BRCA1	HLA-A	KRAS	RASA1
ARID1A	BRCA2	HLA-B	NBN	RECQL4
ARID1B	CDH1	HLA-C	NRAS	ROS1
AXIN1	CDKN2A	IDH1	NSD1	TP53
BARD1	CYLD	INHA	PIK3CA	TP53BP1
BAX	EGFR	KMT2B	POLD1	...
BCOR	ERBB2	KMT2C	QKI	
BRAF	FBXW7	KMT2D	RAC1	

30+ genes 40+ loci (black: 0.5% verified genes, red: dilution multiple verification genes)

NOTES:

I. For AF=0.5%, each site was accurately calibrated using **optimized ddPCR**, with an average single-well copy number greater than *6000* to ensure true positivity and distinguish interference from LOB;

II. For AF=0.05%, the high-frequency sites (e.g. 5% of BRAF p.V600E) were used, and the frequency changes of the high-frequency sites were tested by ddPCR (e.g. BRAF p.V600E diluted 10 fold is 0.5%) to determine the accuracy of the dilution fold, thereby ***indirectly verifying*** the frequency changes of the corresponding sites;

III. For AF=0.005%, the high-frequency sites (e.g. 5% of BRAF p.V600E) were used, and the frequency changes of the high-frequency sites were tested by ddPCR (e.g. BRAF p.V600E diluted 100 fold is 0.05%) to determine the accuracy of the dilution fold, thereby ***indirectly verifying*** the frequency changes of the corresponding sites (for 0.05% of BRAF p.V600E was tested multiple times using optimized ddPCR, with the following requirements: the LOB of the site was controlled below 5 copies, the effective copy number was greater than *12,000*, and the average value was calculated after multiple tests);

IV. For AF=0%, the ddPCR conditions consistent with 0.5% were used, and the positive value was lower than the LOB value while ensuring that the number of copies was greater than 6,000;

CONTACT US

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