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| **Table S1.** Characteristics of the primers and probes as provided by the manufacturer |
| Kit | Chromosome location (hg19) | Amplicon length | COSMIC ID | Nucleotide change of target mutations | Amino acid changes of target mutations | Probe fluorophore  |
| PrimePCR™ ddPCR™ Mutation Detection Assay Kit: *EGFR* WT for p.T790M, and *EGFR* p.T790M, Human | chr7:55249042-55249164 | 80 | COSM6240 | c.2369C>T | p.T790M | FAM/HEX |
| PrimePCR™ ddPCR™ Mutation Detection Assay Kit: *EGFR* WT for p.L858R, and *EGFR* p.L858R, Human | chr7:55259483-55259605 | 73 |  COSM6224 |  c.2573T>G | p.L858R | FAM/HEX |
| PrimePCR™ ddPCR™ *EGFR* Exon 19 Deletions Screening Kit |  (-) |  (-) | COSM13551, COSM6223, COSM6225, COSM12419, COSM6220, COSM51527, COSM12383, COSM12387, COSM12420, COSM6255, COSM12382, COSM6218, COSM12369 and COSM12370 | 15 deletion mutations in exon 19 (c.2235\_2252>AAT, c.2235\_2249del15, c.2236\_2250del15, c.2238\_2252>GCA, c.2238\_2255del18, c.2239\_2253>CAA, c.2239\_2251>C, .2239\_2258>CA, c.2239\_2252>CA, c.2239\_2256del18, c.2239\_2248TTAAGAGAAG>C, c.2239\_2253del15, c.2239\_2247delTTAAGAGAA, c.2240\_2254del15 and c.2240\_2257del18) | p.E746\_T751>I, p.E746\_A750delELREA, p.E746\_A750delELREA, p.L747\_T751>Q , p.E746\_S752>D, p.L747\_T751>Q, p.L747\_T751>P, .L747\_P753>Q, p.L747\_T751>Q , p.L747\_S752delLREATS , p.L747\_A750>P, p.L747\_T751delLREAT, p.L747\_E749delLRE, p.L747\_T751delLREAT and p.L747\_P753>S | FAM/HEX |
| Abbreviations: WT, wild-type.  |

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| **Table S2**. The LOD of the ddPCR assay |
| Sample # | Blanka | 　 | Healthy controlb-cfDNA | 　 | Healthy controlb-exoTNA |
| L858R | Exon 19 deletion (ΔE746 - A750) | T790M  |  | L858R | Exon 19 deletion (ΔE746 - A750) | T790M  |  | L858R | Exon 19 deletion (ΔE746 - A750) | T790M  |
| Wild-type events | Positive type events | Wild-type events | Positive type events | Wild-type events | Positive type events | 　 | Wild-type events | Positive type events | Wild-type events | Positive type events | Wild-type events | Positive type events | 　 | Wild-type events | Positive type events | Wild-type events | Positive type events | Wild-type events | Positive type events |
| #1 | 0 | 0 | 0 | 1 | 0 | 0 |  | 68 | 0 | 59 | 0 | 51 | 0 |  | 58 | 0 | 82 | 0 | 44 | 0 |
| #2 | 0 | 0 | 0 | 0 | 0 | 0 |  | 89 | 0 | 87 | 0 | 101 | 0 |  | 100 | 0 | 53 | 0 | 50 | 0 |
| #3 | 0 | 0 | 0 | 0 | 0 | 0 |  | 334 | 0 | 282 | 0 | 172 | 0 |  | 236 | 0 | 252 | 0 | 156 | 0 |
| #4 | 0 | 0 | 0 | 0 | 0 | 0 |  | 69 | 0 | 55 | 0 | 51 | 0 |  | 66 | 0 | 51 | 0 | 44 | 0 |
| #5 | 3 | 0 | 5 | 0 | 2 | 0 |  | 94 | 1 | 86 | 0 | 101 | 0 |  | 84 | 1 | 119 | 1 | 50 | 0 |
| #6 | 0 | 0 | 1 | 0 | 0 | 0 |  | 374 | 0 | 185 | 0 | 172 | 0 |  | 288 | 0 | 185 | 0 | 156 | 0 |
| #7 | 0 | 0 | 0 | 0 | 0 | 0 |  | 85 | 0 | 46 | 0 | 27 | 0 |  | 38 | 0 | 60 | 0 | 41 | 0 |
| #8 | 0 | 0 | 0 | 0 | 0 | 0 | 　 | 233 | 0 | 373 | 2 | 227 | 0 | 　 | 233 | 0 | 329 | 0 | 69 | 0 |
| Event |  | 0 |  | 1.0  |  | 0  |  |  | 1.0  |  | 2.0  |  | 0  |  |  | 1.0  |  | 1.0  |  | 0.0  |
| Mean |  | 0 |  | 0.1  |  | 0  |  |  | 0.1  |  | 0.3  |  | 0  |  |  | 0.1  |  | 0.1  |  | 0.0  |
| SD |  | 0 |  | 0.3  |  | 0  |  |  | 0.3  |  | 0.7  |  | 0  |  |  | 0.3  |  | 0.3  |  | 0.0  |
| LOB |  | 0 |  | 1  |  | 0  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| LOD (CLSI EP17-A2) | 　 | 　 | 0.5  | 　 | 1.8  | 　 | 0.0  | 　 | 　 | 0.5  | 　 | 1.2  | 　 | 0.0  |
| 95% CI upper bound (one-tail Poisson distribution) | 　 | 　 | 0.4  | 　 | 0.6  | 　 | 0.0  | 　 | 　 | 0.4  | 　 | 0.4  | 　 | 0.0  |
| aAnalysis of blank samples without DNA (n = 8) |
| bEight healthy subjects were anonymized and studied as control samples. |
| Abbreviations: LOD, limit of detection; ddPCR, droplet digital polymerase chain reaction; cfDNA, cell-free DNA; exoTNA, exosomal DNA and RNA; SD, standard deviation; LoB, limit of blank.  |

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| **Table S3**. Analytical sensitivity of the ddPCR assay  |
| Reference Materials | Variant | Expected allele frequency (%)a | Expected copies of wild-type DNA per samplea | Expected copies of mutant DNA per samplea | 1st ddPCR in houseof  | 2nd ddPCR in houseof  |
| Allele frequency (%) | Copies of wild-type DNA per sampleb | Copies of mutant DNA per sample | Allele frequency (%) | Copies of wild-type DNA per sampleb | Copies of mutant DNA per sample |
| 1% Multiplex I cfDNA Reference Standard   (HD778) | L858R | 0.8239  | 3884  | 32  | 1.3225  | 2344  | 31 | 1.0471  | 2483 | 26 |
| Ex19del  | 1.2259  | 2284  | 28  | 1.3123  | 1524 | 20 | 1.6033  | 1684 | 27 |
| T790M  | 1.0081  | 1984  | 20  | 1.1500  | 2087 | 24 | 1.1883  | 2188 | 26 |
| 0.1%  Multiplex I cfDNA Reference Standard (HD779) | L858R | 0.0942  | 4248  | 4  | 0.0368  | 2718  | 1  | 0.0631  | 3171 | 2 |
| Ex19del  | 0.0820  | 2440  | 2  | 0.0538  | 1859 | 1  | 0.1109  | 1803 | 2 |
| T790M  | 0.1361  | 2204  | 3  | 0.0744  | 2688 | 2  | 0.1581  | 2530 | 4 |
| 0.02%  Multiplex I cfDNA Reference Standard (HD779)c | L858R | 0.0219  | 10942  | 2  | 0.0172  | 11604  | 2  | 0.0152  | 13161 | 2  |
| Ex19del  | 0.0213  | 9385  | 2  | 0.0299  | 10039 | 3 | 0.0544  | 9195 | 5 |
| T790M  | 0.0205  | 10242  | 2  | 0.0176  | 11359 | 2 | 0.0092  | 10926 | 1 |
| 0.01%  Multiplex I cfDNA Reference Standard (HD779)c | L858R | 0.0112  | 10099  | 1  | 0.0000  | 11138  | 0  | 0.0000  | 11383 | 0  |
| Ex19del  | 0.0128  | 8901  | 1  | 0.0325  | 9232 | 3 | 0.0140  | 7120 | 1  |
| T790M  | 0.0111  | 9805  | 1  | 0.0115  | 8675 | 1 | 0.0297  | 10118 | 3 |
| 0.005%  Multiplex I cfDNA Reference Standard (HD779)c | L858R | 0.0051  | 20215  | 1  | 0.0077  | 12915  | 1  | 0.0130  | 15400 | 2 |
| Ex19del  | 0.0050  | 17811  | 1  | 0.0080  | 12529 | 1  | 0.0000  | 13877 | 0  |
| T790M  | 0.0057  | 19619  | 1  | 0.0066  | 15154 | 1  | 0.0073  | 13733 | 1 |
| aExpected mutant allele frequency and copy number values of wild-type and mutant DNA measured using ddPCR were provided by the manufacturer. Expected copy numbers of wild-type DNA in spiked reference materials were calculated with measured copy numbers of fragmented healthy control DNA (~200bp). |
| bMean wild-type events of duplicated results at L858R, Ex19del, and T790M were measured using ddPCR. |
| ccfDNA Reference Standards (Horizon Discovery) with 0.1% mutant alleles were serially diluted to wild-type DNAs.  |
| Abbreviations: ddPCR, droplet digital polymerase chain reaction; Ex19del, exon 19 deletion. |

**Figure S1.** Assessment of size-selective target exoNAs related to the sensitivity of *EGFR* mutation testing.

cfDNA, short-length exoNAs (DNA and TNA), and a full-length exoDNA were extracted using different commercial kits. ddPCR was performed with 250, 500, 750, and 1,000 μL plasma samples to assess plasma volume; Ex19del, exon 19 deletion.

**Figure S2.** The distribution of isolated nucleic acids.

The shorter NAs (~ 200 bp long) were more abundant than high-molecular weight DNA in exosomes. **a.** cfDNA was extracted using a MagMAX Cell-Free DNA Isolation Kit, **b.** short-length exoDNA in isolated exosomes from plasma was extracted using a MagMAX Cell-Free DNA Isolation Kit, **c.** short-length exoTNA in isolated exosomes from plasma was extracted using a MagMAX™ Total Nucleic Acid Isolation Kit, **d.** full-length exoDNA in isolated exosomes from plasma was extracted using a QIAamp DNA Blood Mini Kit.