[rs121913507](https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=121913507) *[Homo sapiens]*

ATTTGTGATTTTGGTCTAGCCAGAG[A/T]CATCAAGAATGATTCTAATTATGTG

Chromosome: 4:54733155

Gene: KIT

1. Allele specific primer design on wild type nucleotide of KIT gene

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|  | Forward Primer | Reverse Primer |
| Sequence | GATTTTGGTCTAGCCAGAGA | TGTTCAGCATACCATGCAAAT |
| Length | 20 bp | 21 bp |
| Start | 535 | 759 |
| Tm | 54.6 °C | 59.1 °C |
| GC | 45.0 % | 38.1 % |
| Tm | 51.86 °C | 56.57 °C |
| GC% | 45.0 | 38.1 |
| Self-Dimer ( ΔG) | -4.17 kcal/mol | -8.05 kcal/mol |
| Hairpin ( ΔG) | -0.62 kcal/mol | -1.37 kcal/mol |
| Cross Dimer (ΔG) | -6.84 kcal/mol | |
| Product size | 225 bp | |

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| 2. Allele specific primer design on mutant nucleotide of KIT gene |  |  |  |  |  |

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|  | Forward Primer | Reverse Primer |
| Sequence | GATTTTGGTCTAGCCAGAGT | TGTTCAGCATACCATGCAAAT |
| Length | 20 bp | 21bp |
| Start | 535 | 759 |
| Tm | 53.6 °C | 59.1 °C |
| GC | 45.0 % | 38.1 % |
| Tm | 50.99 °C | 56.57 °C |
| GC% | 45.0 | 38.1 |
| Self-Dimer ( ΔG) | -4.17  kcal/mol | -8.05  kcal/mol |
| Hairpin ( ΔG) | -0.62  kcal/mol | -1.37  kcal/mol |
| Cross Dimer (ΔG) | -6.84  kcal/mol | |
| Product size | 225 bp | |

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| Left Primer 3: | | | | | |
| Sequence: |  | | | | |
| Start:   535 | Length:   20 bp | Tm:   54.6 °C | GC:   45.0 % | ANY:   6.0 | SELF:   4.0 |
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| Right Primer 3: | | | | | |
| Sequence: |  | | | | |
| Start:   759 | Length:   21 bp | Tm:   59.1 °C | GC:   38.1 % | ANY:   5.0 | SELF:   2.0 |
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| Product Size:   225 bp | | Pair Any: 4.0 | Pair End: 1.0 |  |  |

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| **Analysis Results #1: GATTTTGGTCTAGCCAGAGA** | |
| |  |  |  |  | | --- | --- | --- | --- | | Rating | : | 91.0 |  | | Molecular Wt | : | 6172.1 |  | | Tm | : | 51.86 | °C | | GC% | : | 45.0 |  | | GC Clamp | : | 1 |  | | nmol/A260 | : | 5.02 |  | | ug/A260 | : | 31.0 |  | | ΔG | : | -30.99 | kcal/mol | | |  |  |  |  | | --- | --- | --- | --- | | 3' end stability | : | -6.35 | kcal/mol | | ΔH | : | -146.7 | kcal/mol | | ΔS | : | -0.39 | kcal/°K/mol | | 5' end ΔG | : | -6.94 | kcal/mol | | Self Dimer ( ΔG) | : | [-4.17](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol | | Hairpin ( ΔG) | : | [-0.62](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol | | Repeats (# of pairs) | : |  | kcal/mol | | Run (# of bases) | : | [4](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol | |

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| **Analysis Results #2: TGTTCAGCATACCATGCAAAT** | |
| |  |  |  |  | | --- | --- | --- | --- | | Rating | : | 83.0 |  | | Molecular Wt | : | 6389.27 |  | | Tm | : | 56.57 | °C | | GC% | : | 38.1 |  | | GC Clamp | : | 1 |  | | nmol/A260 | : | 4.89 |  | | ug/A260 | : | 31.27 |  | | ΔG | : | -33.64 | kcal/mol | | |  |  |  |  | | --- | --- | --- | --- | | 3' end stability | : | -7.32 | kcal/mol | | ΔH | : | -153.5 | kcal/mol | | ΔS | : | -0.4 | kcal/°K/mol | | 5' end ΔG | : | -6.82 | kcal/mol | | Self Dimer ( ΔG) | : | [-8.05](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol | | Hairpin ( ΔG) | : | [-1.37](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol | | Repeats (# of pairs) | : |  | kcal/mol | | Run (# of bases) | : | [3](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol | |

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| |  |  |  |  | | --- | --- | --- | --- | | Cross Dimer (ΔG) | : | [-6.84](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol | |

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| Pair 2: |  |  |  |  |  |
| Left Primer 2: | | | | | |
| Sequence: |  | | | | |
| Start:   535 | Length:   20 bp | Tm:   53.6 °C | GC:   45.0 % | ANY:   6.0 | SELF:   6.0 |
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| Right Primer 2: | | | | | |
| Sequence: |  | | | | |
| Start:   759 | Length:   21 bp | Tm:   59.1 °C | GC:   38.1 % | ANY:   5.0 | SELF:   2.0 |
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| Product Size:   225 bp | | Pair Any: 4.0 | Pair End: 1.0 |  |  |

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| **Analysis Results #1: GATTTTGGTCTAGCCAGAGT** | |
| |  |  |  |  | | --- | --- | --- | --- | | Rating | : | 91.0 |  | | Molecular Wt | : | 6163.09 |  | | Tm | : | 50.99 | °C | | GC% | : | 45.0 |  | | GC Clamp | : | 1 |  | | nmol/A260 | : | 5.16 |  | | ug/A260 | : | 31.78 |  | | ΔG | : | -30.76 | kcal/mol | | |  |  |  |  | | --- | --- | --- | --- | | 3' end stability | : | -6.11 | kcal/mol | | ΔH | : | -147.6 | kcal/mol | | ΔS | : | -0.39 | kcal/°K/mol | | 5' end ΔG | : | -6.94 | kcal/mol | | Self Dimer ( ΔG) | : | [-4.17](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol | | Hairpin ( ΔG) | : | [-0.62](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol | | Repeats (# of pairs) | : |  | kcal/mol | | Run (# of bases) | : | [4](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol | |

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| **Analysis Results #2: TGTTCAGCATACCATGCAAAT** | |
| |  |  |  |  | | --- | --- | --- | --- | | Rating | : | 83.0 |  | | Molecular Wt | : | 6389.27 |  | | Tm | : | 56.57 | °C | | GC% | : | 38.1 |  | | GC Clamp | : | 1 |  | | nmol/A260 | : | 4.89 |  | | ug/A260 | : | 31.27 |  | | ΔG | : | -33.64 | kcal/mol | | |  |  |  |  | | --- | --- | --- | --- | | 3' end stability | : | -7.32 | kcal/mol | | ΔH | : | -153.5 | kcal/mol | | ΔS | : | -0.4 | kcal/°K/mol | | 5' end ΔG | : | -6.82 | kcal/mol | | Self Dimer ( ΔG) | : | [-8.05](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol | | Hairpin ( ΔG) | : | [-1.37](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol | | Repeats (# of pairs) | : |  | kcal/mol | | Run (# of bases) | : | [3](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol | |

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| |  |  |  |  | | --- | --- | --- | --- | | Cross Dimer (ΔG) | : | [-6.84](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol | |