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CLUSTAL O(1.2.4) multiple sequence alignment


wt_HT1_unmutated_sequence      ------------------------------------------------------------      0
5M_Full_Sequence               GGTCTGCTAGCTGTGGTATGGCTGTGCAGGTCGTAATCACTGCATAATTCGTGTCGCTCA      60


wt_HT1_unmutated_sequence      ------------------------------------------------------------      0
5M_Full_Sequence               AGGCGCACTCCCGTTCTGGATAATGTTTTTTGCGCCGACATCATAACGGTTCTGGCAAAT      120


wt_HT1_unmutated_sequence      ------------------------------------------------------------      0
5M_Full_Sequence               ATTCTGAAATGAGCTGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGC      180


wt_HT1_unmutated_sequence      ------------------------------------------------------------      0
5M_Full_Sequence               GGATAACAATTTCACACAGGAAACAGTATTCATGTCCCCTATATCTAGACATCATCATCA      240


wt_HT1_unmutated_sequence      -------------------------GCATCCCTACGCGCCAATGATGCACCGATTGTGCT      35
5M_Full_Sequence               TCATCATCTGGTTCCGCGTGGATCCGCATCCCTACGCGCCAATGATGCACCGATTGTGCT      300
                                                        ***********************************

wt_HT1_unmutated_sequence      TCTCCATGGGTTTACCGGATGGGGACGAGAGGAAATGTTTGGATTCAAGTATTGGGGCGG      95
5M_Full_Sequence               TCTCCATGGGTTTACCGGATGGGGACGAGAGGAAATGTTTGGATTCAAGTATTGGGGCGG      360
                               ************************************************************

wt_HT1_unmutated_sequence      CGTGCGCGGCGATATCGAACAATGGCTGAACGACAACGGTTATCGAACGTATACGCTGGC      155
5M_Full_Sequence               CGTGCGCGGCGATATCGAACAATGGCTGAACGAGAACGGTTATCGAACGTATACGCTGGC      420
                               ********************************* **************************

wt_HT1_unmutated_sequence      GGTCGGACCGCTCTCGAGCAACTGGGACCGGGCGTGTGAAGCGTATGCTCAGCTTGTCGG      215
5M_Full_Sequence               GGTCGGACCGCTCTCGAGCAACTGGGACCGGGCGTGTGAAGCGTATGCTCAGCTTGTCGG      480
                               ************************************************************

wt_HT1_unmutated_sequence      CGGGACGGTCGATTATGGGGCAGCCCATGCGGCAAAGCACGGCCATGCGCGGTTTGGCCG      275
5M_Full_Sequence               CGGGACGGTCGATTATGGGGCAGCCCATGCGGCAAAGCACGGCCATGCGCGGTTTGGCCG      540
                               ************************************************************

wt_HT1_unmutated_sequence      CACTTATCCCGGCCTGTTGCCGGAATTGAAAAGGGGTGGCCGCATCCATATCATCGCCCA      335
5M_Full_Sequence               CACTTATCCCGGCCTGTTGCCGGAATTGAAAAGGGGTGGCCGCATCCATATCATCGCCCA      600
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wt_HT1_unmutated_sequence      CAGCCAAGGGGGGCACACGCCCCGCATGCTTGTCTCGCTCCTAGAGAACGGAAGCCAAGA      395
5M_Full_Sequence               CAGCCAAGGGGGGCACAACCCCCGCATGCTTGTCTCGCTCCTAGAGAACGGAAGCCAAGA      660
                               *****************  *****************************************

wt_HT1_unmutated_sequence      AGAGCGGGAGTACGCCAAGGCGCATAACGTGTCGTTGTCACCGTTGTTTGAAGGTGGACA      455
5M_Full_Sequence               AGAGCGGGAGTACGCCAAGGCGCATAACGTGTCGTTGTCACCGTTGTTTGAAGGTGGACA      720
                               ************************************************************

wt_HT1_unmutated_sequence      TCATTTTGTGTTGAGTGTGACGACCATCGCCACTCCTCATGACGGGACGACGCTTGTCAA      515
5M_Full_Sequence               TCATTTTGTGTTGAGTGTGACGACCATCGCCACTCCTCATGACGGGACGACGCTTGTCAA      780
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wt_HT1_unmutated_sequence      CATGGTTGATTTCACCGATCGCTTTTTTGACTTGCAAAAAGCGGTGTTGGAAGCGGCGGC      575
5M_Full_Sequence               CATGGTTGATTTCACCGATCGCTTTTTTGACTTGCAAAAAGCGGTGTTGGAAGCGGCGGC      840
                               ************************************************************

wt_HT1_unmutated_sequence      TGTCGCCAGCAACGTGCCGTACACGAGTCAAGTATACGATTTTAAGCTCGACCAATGGGG      635
5M_Full_Sequence               TGTCGCCAGCAACGTGCCGTACACGAGTCAAGTATACGATTTTAAGCTCGACCAATGGGG      900
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wt_HT1_unmutated_sequence      ACTGCGCCGCCAGCCGGGTGAATCGTTCGACCATTATTT GAA GGCTCAAGCGCTCCCC     695
5M_Full_Sequence               ACTGCGCCGCCAGCCGGGTGAATCGTTCGACCATTATTT GAT GGCTCAAGCGCTCCCC     960
                               *************************************** **   ****************

wt_HT1_unmutated_sequence      TGTTTGGACGTCCACAGATACCGCCCGCTACGATTTATCCGTTTCCGGAGC GAG AGTT     755
5M_Full_Sequence               TGTTTGGACGTCCACAGATACCGCCCGCTACGATTTATCCGTTTCCGGAGC TTG AGTT     1020
                               ***************************************************   * ****

wt_HT1_unmutated_sequence      GAATCAATGGGTGCAAGCAAGCCCGAATACGTATTATTTGAGTTTCTCTACAGAACGGAC     815
5M_Full_Sequence               GAATCAATGGGTGCAAGCAAGCCCGAATACGTATTATTTGAGTTTCTCTACAGAACGGAC     1080
                               ************************************************************

wt_HT1_unmutated_sequence      GTATCGCGGAGCGCTCACAGGCAACCATTATCCCGAACTCGGAATGAATGCATTCAGCGC     875
5M_Full_Sequence               GTATCGCGGAGCGCTCACAGGCAACCATTATCCCGAACTCGGAATGAATGCATTCAGCGC     1140
                               ************************************************************

wt_HT1_unmutated_sequence      GGTCGTATGCGCTCCGTTTCTCGGTTCGTACCG AAT CGACGCTCGGCATTGACGACCG     935
5M_Full_Sequence               GGTCGTATGCGCTCCGTTTCTCGGTTCGTACCG GAG CGACGCTCGGCATTGACGACCG     1200
                               *********************************  *  **********************

wt_HT1_unmutated_sequence      ATGGTTGGAGAACGATGGCATTGTCAATACGGTTTCCATGAACGGTCCAAAGCGTGGATC     995
5M_Full_Sequence               ATGGTTGGAGAACGATGGCATTGTCAATACGGTTTCCATGAACGGTCCAAAGCGTGGATC     1260
                               ************************************************************

wt_HT1_unmutated_sequence      AAGCGATCGGATCGTGCCGTATGACGGGACGTTGAAAAAAGGGGTTTGGAATGATATGGG     1055
5M_Full_Sequence               AAGCGATCGGATCGTGCCGTATGACGGGACGTTGAAAAAAGGGGTTTGGAATGATATGGG     1320
                               ************************************************************

wt_HT1_unmutated_sequence      AACGTACAACGTCGACCATTTGGAAATCATCGGCGTTGACCCGAATCCGTCATTTGATAT     1115
5M_Full_Sequence               AACGTACAACGTCGACCATTTGGAAATCATCGGCGTTGACCCGAATCCGTCATTTGATAT     1380
                               ************************************************************

wt_HT1_unmutated_sequence      TCGCGCCTTTTATTTGCGGCTTGCCGAGCAGTTGGCGAGCTTGCAGCCTTAA--------     1167
5M_Full_Sequence               TCGCGCCTTTTATTTGCGGCTTGCCGAGCAGTTGGCGAGCTTGCAGCCTTAAGAATTCCC     1440
                               ****************************************************

wt_HT1_unmutated_sequence      ------------------------------------------------------------     1167
5M_Full_Sequence               GGGTCGACTCGAGCGGCCGCATCGTGACTGACTGACGATCTGCCTCGCGCGTTACGTATA     1500


wt_HT1_unmutated_sequence      -           1167
5M_Full_Sequence               G           1501
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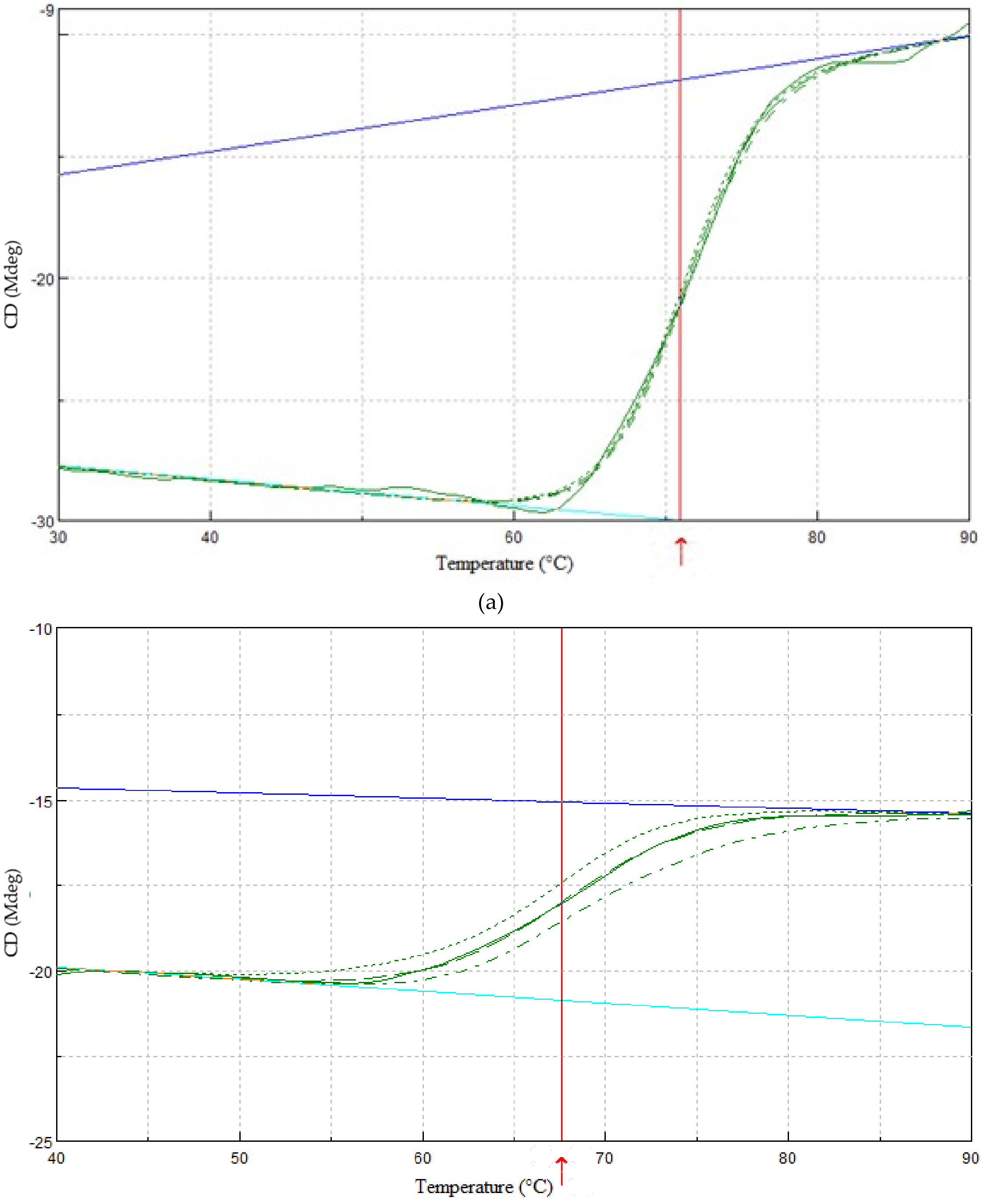
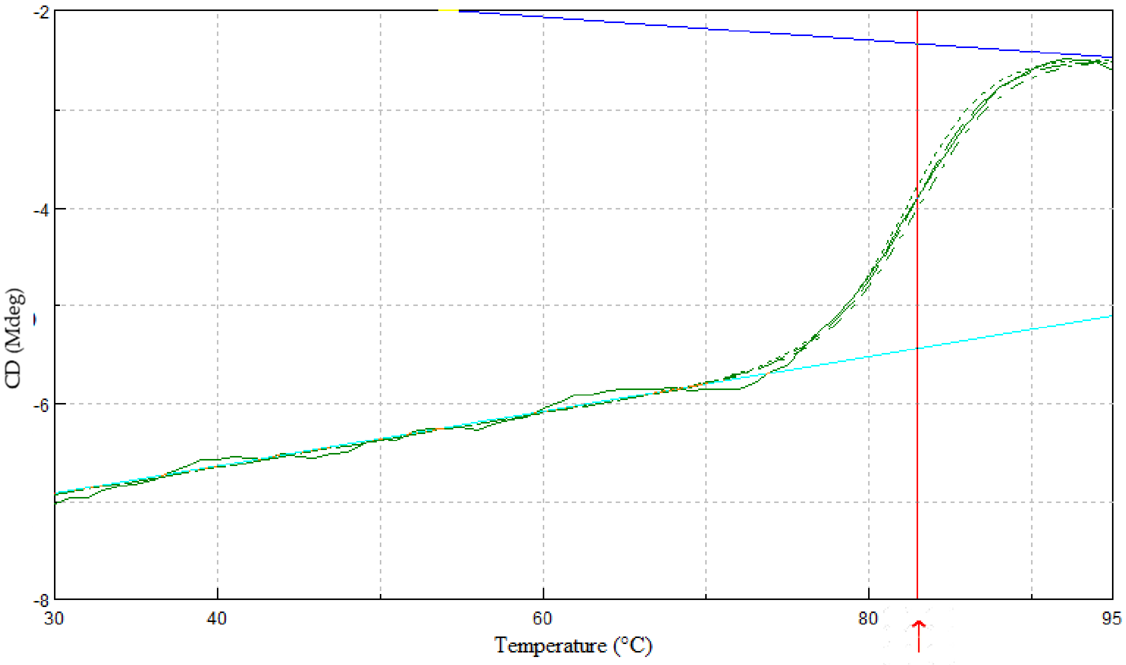
Figure S1. DNA sequence alignments of 5M mutated lipase and original sequence of T1 lipase for verification of mutation. The multiple sequences alignment was generated from https://www.ebi.ac.uk/Tools/services/rest/clustalo/result/clustalo-I20190408-165909-0083-17877188-p2m/aln-clustal_num. The mutated sequences are marked in red boxes.



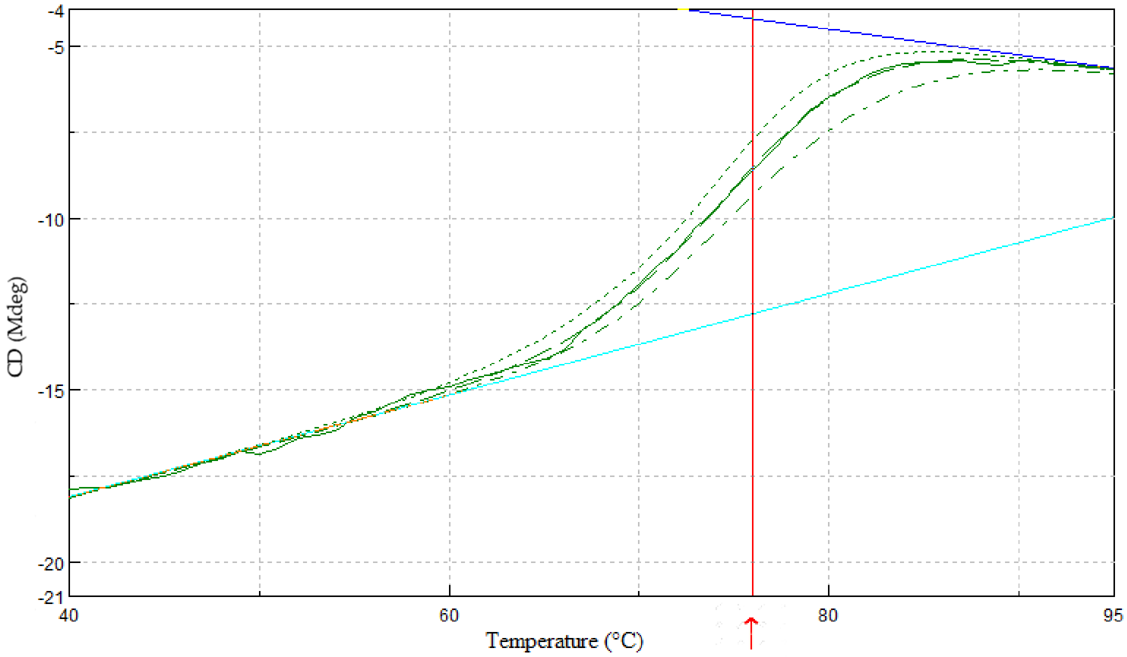
(a)

(b)

Figure S2. Melting temperature (T_m) curve of wt-HT1 and 5M mutant lipases using Circular Dichroism without calcium ion. (a) Temperature profile of wt-HT1 lipase. (b) Temperature profile of 5M mutant lipase. Red arrow indicated the thermal denaturation point of lipases.



(a)



(b)

Figure S3. Melting temperature (T_m) curve of wt-HT1 and 5M mutant lipases using Circular Dichroism with the presence of 1 mM calcium ion. (a) Temperature profile of wt-HT1 lipase with 1 mM calcium ion. (b) Temperature profile of 5M mutant lipase with 1 mM calcium ion. Red arrow indicated the thermal denaturation point of lipases.

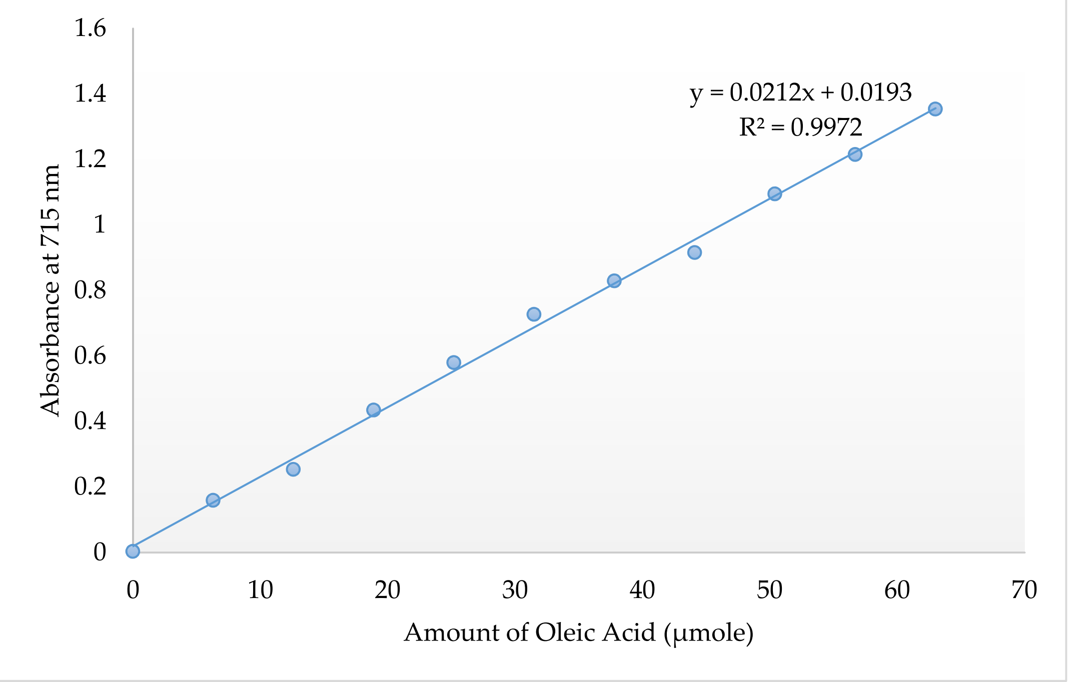


Figure S4. Oleic acid standard curve based on the method of Kwon and Rhee [59].