

# Screening and Identifying a Novel ssDNA Aptamer against Alpha-fetoprotein Using CE-SELEX

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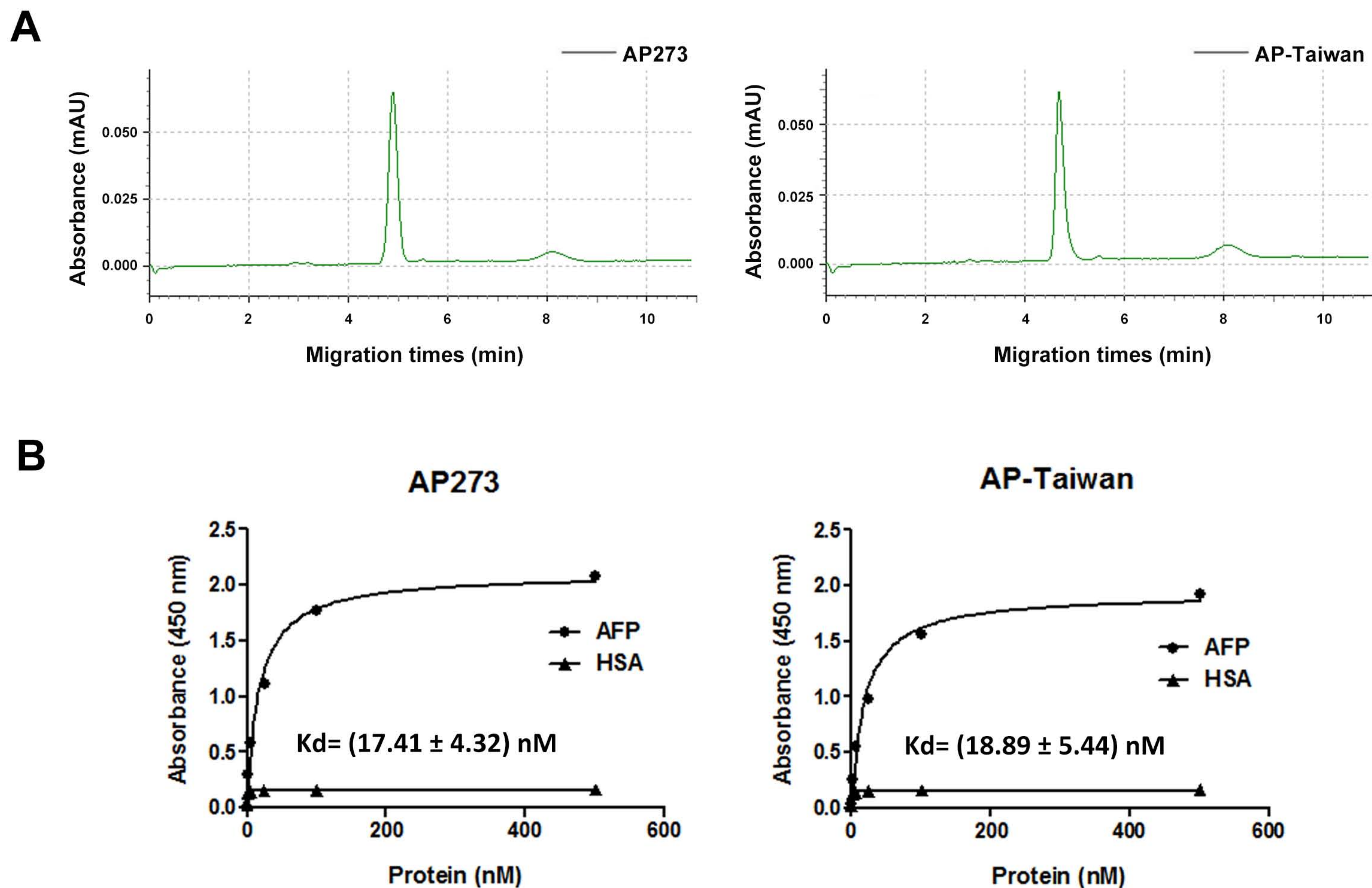
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AP202	GTGACGCTCCTAACGCTGAC	GGTCTTTTCC. CTGACTTAATCTCATCTTTTCCGGC	CTGTCCGTCCGAACCAATC	75
AP204	GTGACGCTCCTAACGCTGAC	AACGGACGAT. CAAATCTCTATAGGCTGATTATAC	CTGTCCGTCCGAACCAATC	75
AP205	GTGACGCTCCTAACGCTGAC	GGCGGTGCAT. GATCCCTAGGACAGGGTTTCCAGC	CTGTCCGTCCGAACCAATC	75
AP206	GTGACGCTCCTAACGCTGAC	TTTTAATTCT. TGTTATTGGTATATCCGCCTTGAC	CTGTCCGTCCGAACCAATC	75
AP208	GTGACGCTCCTAACGCTGAC	AGGAACGCC. CCTAGGTACAGAGCAGGAGTGCAT	CTGTCCGTCCGAACCAATC	75
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AP210	GTGACGCTCCTAACGCTGAC	CAAGCATGGT. GGGTGTGTCGTCCTCATCTCCTTC	CTGTCCGTCCGAACCAATC	75
AP211	GTGACGCTCCTAACGCTGAC	TTTTTGGGAA. TGCGTATGTATGCTGCCACGTACG	CTGTCCGTCCGAACCAATC	75
AP212	GTGACGCTCCTAACGCTGAC	TAAGTAGTGC. GACTGAAGTCAATGAGAGGTGCGT	CTGTCCGTCCGAACCAATC	75
AP214	GTGACGCTCCTAACGCTGAC	TCACGGGGCC. TACCGAAATCTAGGCTGCCACATG	CTGTCCGTCCGAACCAATC	75
AP215	GTGACGCTCCTAACGCTGAC	TGTATTCTGC. AGTCTTATTCAAGTCGGTCCGAATGAC	CTGTCCGTCCGAACCAATC	75
AP216	GTGACGCTCCTAACGCTGAC	GGGAATAACG. TGTAACGTAATAGTGGGTTCACCT	CTGTCCGTCCGAACCAATC	75
AP217	GTGACGCTCCTAACGCTGAC	CGCGAGCCAC. CGACGGACACTCGGAATCCATTATT	CTGTCCGTCCGAACCAATC	75
AP218	GTGACGCTCCTAACGCTGAC	CACAGGTAGG. GACAACATCACATGGGCGTTAGATAC	CTGTCCGTCCGAACCAATC	75
AP219	GTGACGCTCCTAACGCTGAC	CCGCGGTTCC. ATCTTGTATGCCTGTGGATGTTT	CTGTCCGTCCGAACCAATC	75
AP220	GTGACGCTCCTAACGCTGAC	TGTCGGAAAA. TAGCCAACACTTCCGCAGCGTGTGT	CTGTCCGTCCGAACCAATC	75
AP221	GTGACGCTCCTAACGCTGAC	AACACCCTAA. TACCCACGGGTTGCCATACGGGGGT	CTGTCCGTCCGAACCAATC	75
AP222	GTGACGCTCCTAACGCTGAC	GGCACGAAGA. TGCTGTGAGGCCCTTTCGCTGTGCA	CTGTCCGTCCGAACCAATC	75
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AP224	GTGACGCTCCTAACGCTGAC	CGTCAGTATG. GTTCTTGAAGTATGTGACTGCTGG	CTGTCCGTCCGAACCAATC	75
AP225	GTGACGCTCCTAACGCTGAC	AGGTGACCT. GTTGGAAAGTTAGGCGTCTTAAAC	CTGTCCGTCCGAACCAATC	75
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AP228	GTGACGCTCCTAACGCTGAC	CTTTTACGTG. GTGGTAGTATACTTTAATGTAATTT	CTGTCCGTCCGAACCAATC	75
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AP230	GTGACGCTCCTAACGCTGAC	GACACACCTT. CGAAGCCACGAGAATGGATGACT	CTGTCCGTCCGAACCAATC	75
AP231	GTGACGCTCCTAACGCTGAC	AAATGGGCAA. AGCGTCTAAAGAACTTGGTAGGGT	CTGTCCGTCCGAACCAATC	75
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AP237	GTGACGCTCCTAACGCTGAC	GGTGGTAAGA. AGTAGCTTACGGCTTGTGTTTAT	CTGTCCGTCCGAACCAATC	75
AP238	GTGACGCTCCTAACGCTGAC	CTGCGCTCGA. CGTAGTCCGCTCGGGAGTACCAGGC	CTGTCCGTCCGAACCAATC	75
AP239	GTGACGCTCCTAACGCTGAC	AGGAGACACA. TCTTAAAGCTGCCACGCCGATCC	CTGTCCGTCCGAACCAATC	75
AP240	GTGACGCTCCTAACGCTGAC	TGAAAGGGG. FTACCTTAAAGGACTAGCGTCACTG	CTGTCCGTCCGAACCAATC	75
AP241	GTGACGCTCCTAACGCTGAC	CGGGGTGAAT. CTCATGCTTACGGGGTTCGACTAG	CTGTCCGTCCGAACCAATC	75
AP242	GTGACGCTCCTAACGCTGAC	ATAGCGCACT. GTAGTAGGGACACCACCTCCGCGAG	CTGTCCGTCCGAACCAATC	75
AP243	GTGACGCTCCTAACGCTGAC	TCGGGGACAT. ACGCGGGCGAAGTGAACGTGTGT	CTGTCCGTCCGAACCAATC	75
AP244	GTGACGCTCCTAACGCTGAC	GGCCCGTCAA. TCCCGCGTACTCATTGGTCCCTTAC	CTGTCCGTCCGAACCAATC	75
AP246	GTGACGCTCCTAACGCTGAC	CAGCACTGTC. GGATTAGCCACTGTTAGTTGGAGCC	CTGTCCGTCCGAACCAATC	75
AP248	GTGACGCTCCTAACGCTGAC	TGTCGTTTGT. TTAGTGGATATTTCTTCTCTCCG	CTGTCCGTCCGAACCAATC	75
AP249	GTGACGCTCCTAACGCTGAC	GTAAGAGCAC. CTTTAAAGTACAGTACATGCCAGGG	CTGTCCGTCCGAACCAATC	75
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AP251	GTGACGCTCCTAACGCTGAC	TCAGAACGAC. CCGGTTCTGCTACTGGCTGGTGGG	CTGTCCGTCCGAACCAATC	75
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AP254	GTGACGCTCCTAACGCTGAC	TTTGGAGAGG. CGTCGCCATGTTCCACTTAGCGTGG	CTGTCCGTCCGAACCAATC	75
AP255	GTGACGCTCCTAACGCTGAC	TTTGGAGAGG. CGTCGCCATGTTCCGCTTAGCGTGG	CTGTCCGTCCGAACCAATC	75
AP256	GTGACGCTCCTAACGCTGAC	CAAGTGCAT. AATTCCGTCAAATTCGGAAGGTAGG	CTGTCCGTCCGAACCAATC	75
AP257	GTGACGCTCCTAACGCTGAC	GGTCTCAGG. TACCGCCAGCTTGTTCAGACGACT	CTGTCCGTCCGAACCAATC	75
AP258	GTGACGCTCCTAACGCTGAC	GTGGGCGTCG. AATTGTCCGTCGTGATCTGGTCGCAC	CTGTCCGTCCGAACCAATC	75
AP260	GTGACGCTCCTAACGCTGAC	GAGTCAAGTT. ATGTCGTACAGCGGAGCGGATTTCC	CTGTCCGTCCGAACCAATC	75
AP261	GTGACGCTCCTAACGCTGAC	GGCGGGGACC. TTGATCATACTTTCGTTCCGAATCTC	CTGTCCGTCCGAACCAATC	75
AP262	GTGACGCTCCTAACGCTGAC	CAGCGGGATT. ATTCCCTCTTTGGGGCGGATCGACAC	CTGTCCGTCCGAACCAATC	75
AP263	GTGACGCTCCTAACGCTGAC	GAGTTGACTA. CAGATGGGCCTTAGGGTAGCTTTAC	CTGTCCGTCCGAACCAATC	75
AP264	GTGACGCTCCTAACGCTGAC	CATAAGACAT. AGTAGCCCGGATTTAGCTCGGGT	CTGTCCGTCCGAACCAATC	75
AP265	GTGACGCTCCTAACGCTGAC	TAAGTGTGGT. CTAAGCTGTCTCTGGCTCAGTATC	CTGTCCGTCCGAACCAATC	75
AP266	GTGACGCTCCTAACGCTGAC	GGCCCGTTG. GATTACATCCAGGAGCTTAAATTA	CTGTCCGTCCGAACCAATC	75
AP267	GTGACGCTCCTAACGCTGAC	AAATACCTGCG. TTGCTTCTCATGTTGGTCTTTTACAC	CTGTCCGTCCGAACCAATC	75
AP268	GTGACGCTCCTAACGCTGAC	CTCTGTAAGA. AAGAGCGTTCTACAGCCATTTACG	CTGTCCGTCCGAACCAATC	75
AP270	GTGACGCTCCTAACGCTGAC	GGGAGATGGT. TACATATTCACTTTC. CAGCATCT	CTGTCCGTCCGAACCAATC	75
AP271	GTGACGCTCCTAACGCTGAC	TGAGCAGTCC. AAGCCACTACATGTTCCGGTATCTTT	CTGTCCGTCCGAACCAATC	75
AP272	GTGACGCTCCTAACGCTGAC	GGTATTCTTA. ACGTCCGCGTGTATTAGTCTTATTGTT	CTGTCCGTCCGAACCAATC	75
AP273	GTGACGCTCCTAACGCTGAC	TCAGGTGCAG. TTCTCGACTCGTCTTGTATGTGGGT	CTGTCCGTCCGAACCAATC	75
AP274	GTGACGCTCCTAACGCTGAC	CTGGTGACCT. GCGTTTATATCAGTACAGCCGTCGC	CTGTCCGTCCGAACCAATC	75
AP276	GTGACGCTCCTAACGCTGAC	GCAGGGGCCA. GTTTTGTACGTCATCTGGCGGGCC	CTGTCCGTCCGAACCAATC	75
AP277	GTGACGCTCCTAACGCTGAC	AAC TGGGCTG. CGTCTTCCCGTCTCACTGCAATC	CTGTCCGTCCGAACCAATC	75
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AP281	GTGACGCTCCTAACGCTGAC	GATGGTCAGGACTGGTGAATCAGTTTGT. TGG	CTGTCCGTCCGAACCAATC	75
AP282	GTGACGCTCCTAACGCTGAC	GGCCCTAGTG. ACCTGGGGTTCAGTTCTCGCTCAG	CTGTCCGTCCGAACCAATC	75
AP283	GTGACGCTCCTAACGCTGAC	CAGGGCATTG. CCGGAGCACTAGGGGTAGTCTTGG	CTGTCCGTCCGAACCAATC	75
AP284	GTGACGCTCCTAACGCTGAC	TGTGCACAGC. CTATGCTCAGGCAACTTAGGCAG	CTGTCCGTCCGAACCAATC	75
AP285	GTGACGCTCCTAACGCTGAC	CAGACATTTA. CTGGGATCGATAAAGATCGTCTGAC	CTGTCCGTCCGAACCAATC	75
AP287	GTGACGCTCCTAACGCTGAC	CTTGTAGTGC. GTAGGGATCGAGTACAGACTCAGC	CTGTCCGTCCGAACCAATC	75
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AP290	GTGACGCTCCTAACGCTGAC	GGCTGAGAAC. CTGCTGCTTACTTAGACCATCGGT	CTGTCCGTCCGAACCAATC	75
AP292	GTGACGCTCCTAACGCTGAC	GACTTCTGCT. AACACACGGTCAGGCGTTGCTCCTA	CTGTCCGTCCGAACCAATC	75
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Consensus gtgacgctcctaacgctgac

cctgtcgtccgaaccaatc

Supplemental Figure 1. Complete sequence alignment of 83 aptamers using DNAMAN





**Supplemental Figure 2. Binding affinity evaluation of AP273 and AP-Taiwan**

(A) Comparison between AP273 and AP-Taiwan using CE assays. Both of these aptamers run the similar peaks. (B) The microplate testing analysis of the comparison between AP273 and AP-Taiwan. The 96-well microplate was coated with AP273 or AP-Taiwan and pre-blocked with non-specific aptamers. A volume of 100  $\mu$ l of AFP or human serum albumin (HSA) protein (as the negative control) of different concentrations (500 nM, 100 nM, 25 nM, 5 nM, 1 nM, 0 nM) was added to each well and incubated for 1h. After blocking with 3% BSA for 2 h, the plate was incubated with bio-labeled Anti-AFP antibody for 1 h. After washing 3 times, the streptavidin-horseradish peroxidase (SA-HRP) was added and incubated for 30 min, followed by washing. The plate was then added to substrate TMB for colorimetric detection on 450 nm. The titration curves were obtained based on above data and the equation  $Y = B_{max}X / (K_d + X)$  was used to calculate the  $K_d$  according to GraphPad Prism 5.0. Y represented the mean value of OD450 nm,  $B_{max}$  was the maximal value of OD450 nm, and X was the concentration of the protein. Compared to HSA, AP273 and AP-Taiwan all can bind AFP specifically and the  $K_d$  values with AFP were  $17.41 \pm 4.32$  nM and  $18.89 \pm 5.44$  nM, respectively.



