

Supporting information for One-Step Immunoassay for Tetrabromobisphenol A Using a Camelid Single Domain Antibody-Alkaline Phosphatase Fusion Protein

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Figure S-1. Chemical structures of TBBPA and its haptens T1–T6 ¹. T5 conjugated with thyroglobulin was used to immunize the alpaca; T3 conjugated to bovine serum albumin (BSA) was used as the coating antigen for VHH selection.

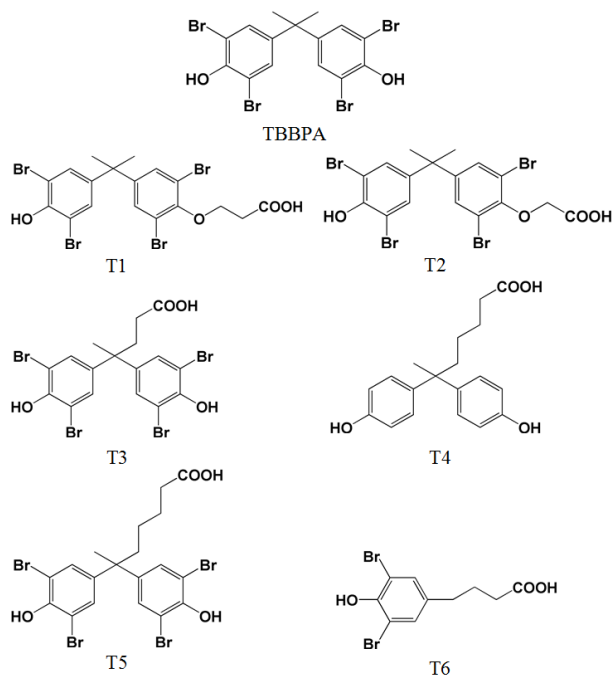


Figure S-2. Amino acid sequences of positive VHHs against TBBPA. Five groups were obtained from 16 clones and one VHH was selected from each group. The dots indicate that the sequences are identical to the T3-4 arbitrarily chosen as a reference sequence. Dashed are marked as the absence of amino acid in comparison with the reference sequence. The sequence of T3-15 was shown in a previous study².

	FR1	CDR1	FR2
T3-4	QLQLVESGGGLVQAGGSLTLS	CVASGSISSIIYPMGWYRQAPGKERELVAA	
T3-9A.....F.....PA.....Q.....	
T3-12P.....R.....A.....RT.....F.....T.....	
T3-15V.....P.....R.....A.....R.....F.....T.....T.....P.....RG.....	
T3-16V.....R.....A.....N.....FR.....NA.....V.....	

	CDR2	FR3
T3-4	VSSSTGGTTYADSVKGRFTIS	---RDYAKNTVYLLQMSSLKPEETAVYYCTY
T3-9	KT.G.Y.....R.....	---N.....LS.....N.....D.....M.....A
T3-12	I.PGST.....	---N.....M.....NN.E.....D.....M.....A
T3-15	SN.F.T.....N.....A.....	---N.....N.....N.....D.....M.....A
T3-16	YRRG.I.....	---TIS.....N.....N.....N.....D.....M.....VA

	CDR3	FR4	HINGE	His tag
T3-4	RG....TARYWGQGTQVTVSS	EPKTPKPKQDQGAGQH	HHHHH	
T3-9	KYG...GVS.D.....	
T3-12	.DRSDA.I.L.....	
T3-15	.DRSDA.I.V.....	
T3-16	.YSLRPEDA.....	AHHS.ED.-H.....	

Figure S-3. SDS-PAGE gel image of purified VHH and VHH-AP. Lane 1: protein standards. Lane 2, 4, 6, 8, and 10 indicate purified VHH T3-4, T3-9, T3-12, T3-15, and T3-16, respectively. Lane 3, 5, 7, 9, and 11 indicate purified T3-4-AP, T3-9-AP, T3-12-AP, T3-15-AP, and T3-16-AP, respectively.

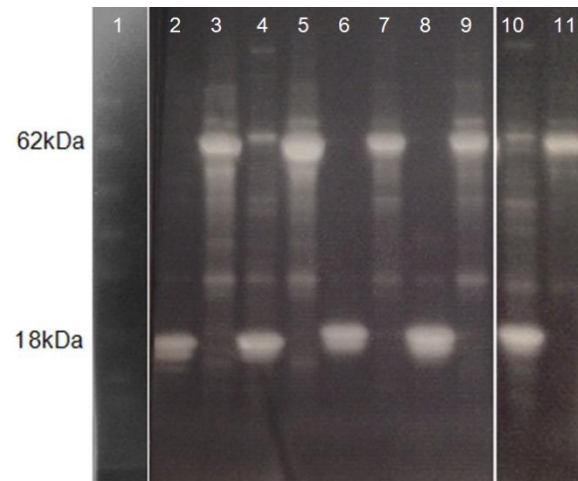


Figure S-4. Calibration curves of T3-15-AP for TBBPA in the one-step assay coated with T3-BSA and T5-BSA, respectively. A series of concentrations of TBBPA were diluted in 10% of methanol in PBS. Values are the mean \pm standard deviations of three well replicates.

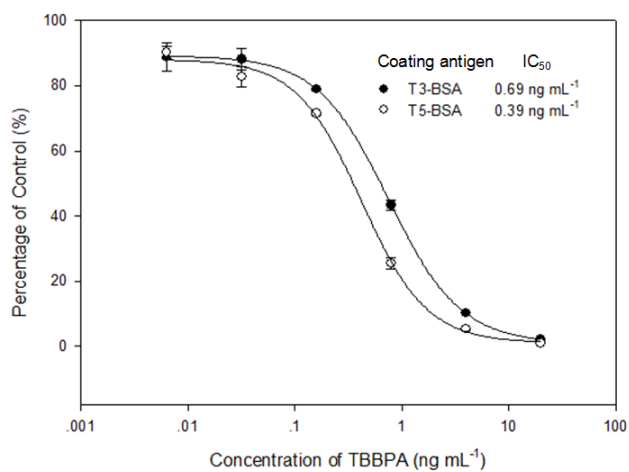


Figure S-5. Comparison of stability between VHH T3-15 (triangle) and T3-15-AP (diamond). A: The tolerances of T3-15 and T3-15-AP on heating at 90 °C. B: The storage stability of T3-15 and T3-15-AP at ambient temperature (roughly 22 °C).

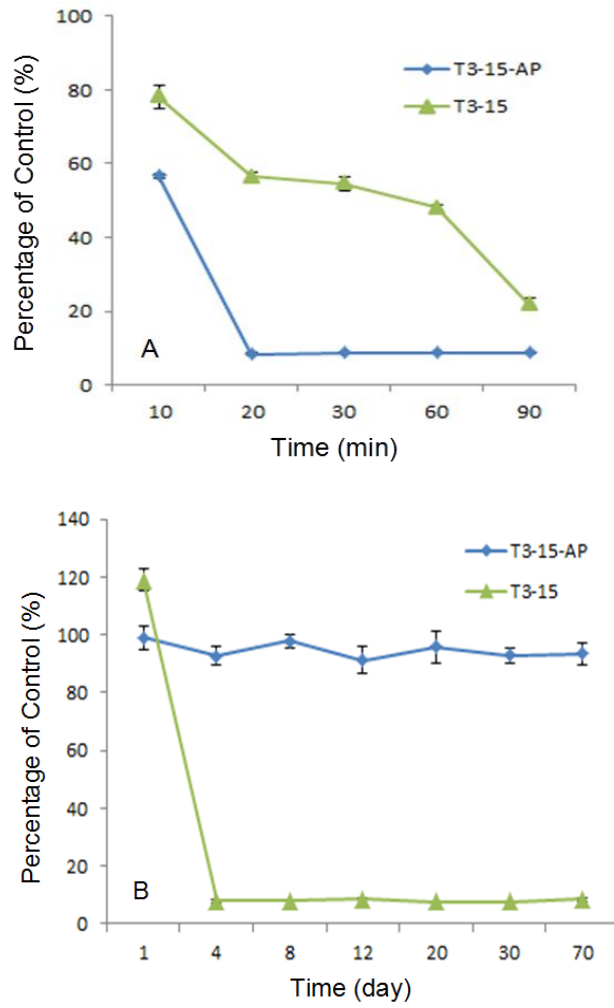
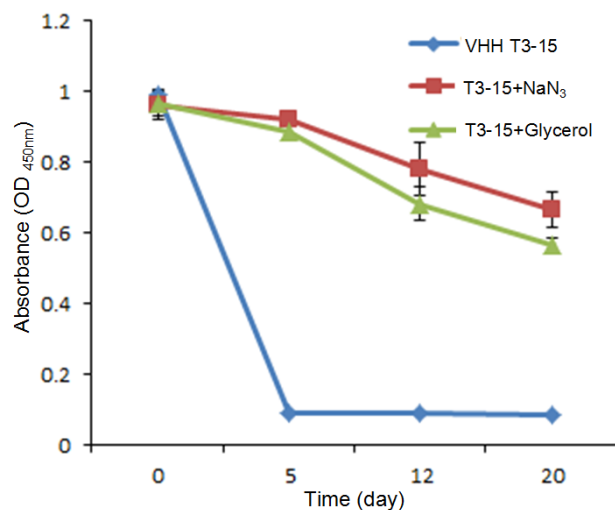


Figure S-6. Binding ability of VHH T3-15, under the protection of reagents NaN_3 and glycerol at ambient temperature, to coating antigen T3-BSA. The final contents of NaN_3 and glycerol in VHH solution were 0.02% and 50%, respectively.



References

1. Xu, T.; Wang, J.; Liu, SZ.; Lu, C.; Shelver, W. L.; Li, Q. X.; Li, J. *Anal Chim Acta*. **2012**, 751, 119–127.
2. Wang, J.; Bever, C. R. S.; Majkova, Z.; Dechant, J. E.; Yang, J.; Gee, S. J.; Xu, T.; Hammock, B. D. *Anal. Chem.* **2014**, 86, 8296–8302.