

Supplementary Information

Heterologous antigen selection of chicken single-chain variable fragment against thiamethoxam

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Table S1. Primers used in this study.

Primers	Sequences (5'-3')
HF- <i>Sfi</i> I	CATGCCATGACTGTGGCCCAGGCGGCCGTGA GTTGGACG
HR-linker	CAGAGCCACCTCCGCCTGAACCGCCTCCAC CGGAGGAGACGATGACTTCGG
LF-linker	TTCAGGCGGAGGTGGCTCTGGCGGTGGCGGTCG GCGCTGACTCAGCCGTCCT
LR- <i>Sfi</i> I	CATGCCATGACTGTGGCCGGCCTGGCCACCTAG AGGTCAGGG
F-AP- <i>Sfi</i> I	CATGCCATGACTGTGGCCCAGCCGGCCGTG AGTTGGACG
R-AP- <i>Sfi</i> I	CATGCCATGACTCGCGGGCCCCGAGGCACCTA GAGGTCAGGG
F-Biotin- <i>Sac</i> I	CGAGCTCCACCATCACCATCACCATGTGACGT TGGACGAGTCCGG
R-Biotin- <i>Hind</i>	CAAGCTTATGGTGATGGTGATGGT
III	GCTGGCCG

Highlight in blue color indicates restriction enzyme sites.

Fig. S1. Structure of Thi, Thi-3C, and Thi-5C.

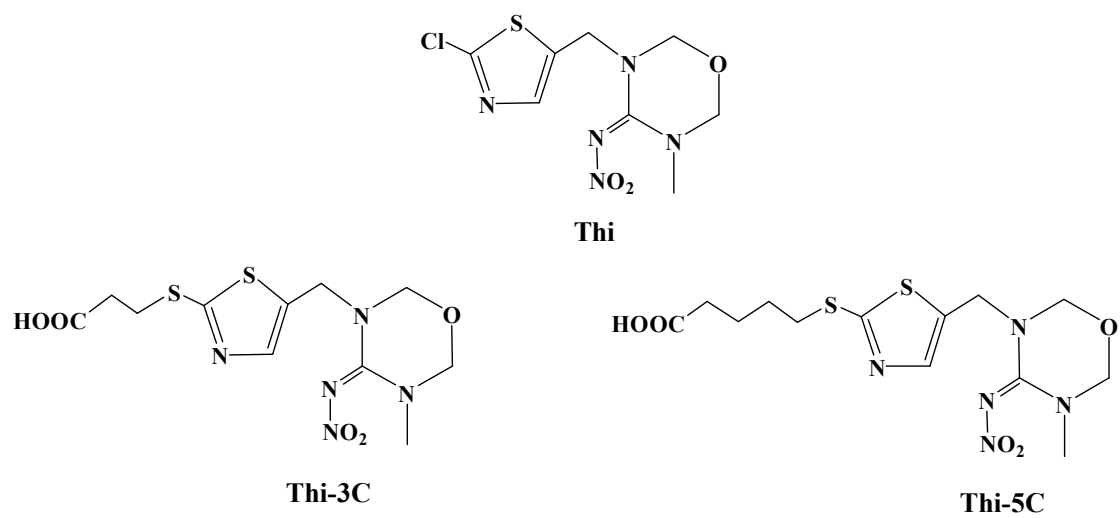


Fig. S2. The amino acid sequences of scFv Thi3-1, Thi2-7, and Thi4-8.

	HFR1	HCDR1	HFR2	HCDR2
Thi3-1	AVTLDESGGGLQTPGGGLSLICKASGFDFS-	TYAVG-WVRQAPGKGLEWVA-SIYSGSYTWYAT		
Thi2-7	AVTLDESGGGLQTPGGALSLVCKASGFDFS-	SHSVA-WVRQAPGKGLEWVA-SIYSGSYTWYAP		
Thi4-8	AVTLDESGGGLQTPGGGLSLICKASGFDFS-	TYAVG-WVRQAPGKGLEWVA-SIYSGSYTWYAT		
	HFR3	HCDR3	HFR4	
Thi3-1	AVKG-RATISKDNGQSTVRLQLNNLRAEDTATYYCTK-	CAYSGCVHRIDA-WGHGTEVIVSS-G		
Thi2-7	AVKG-RATISRDNGQSTVRLQLNNLRAEDTGTYCTK-	CAYSACVHRIDA-WGHGTEVIVSS-G		
Thi4-8	AVKG-RATISKDNGQSTVRLQLNNLRAEDTATYYCTK-	CAYSGCVHRIDA-WGHGTEVIVSS-G		
	Linker	LFR1	LCDR1	LFR2
Thi3-1	GGGSGGGSGGGGS-	ALTQPSSVSANPGETVKITC-SGSSSGN-WYGWYQQKSPGSAPVTVIY-		
Thi2-7	GGGSGGGSGGGGS-	ALTQPSSVSANPGETVEITC-SGGGSS-YYGWYQQKSPGSAPVTVIY-		
Thi4-8	GGGSGGGSGGGGS-	ALTQPSSVSANPGETVKITC-SGSSSGN-WYGWYQQKSPGSAPVTVIY-		
	LCDR2	LFR3	LCDR3	LFR4
Thi3-1	RNNQRPS-NIPSRFSGSLSGSTNTLTITGVQVEDEAVYYC-G-----SIDN-----FGAGTTLTVL			
Thi2-7	SNNKRPS-DIPSRFSGSKSGSTATLTITGVQVEDEAVYFC-GDYDISADSGI-FGAGTTLTVL			
Thi4-8	RNNQRPS-NIPSRFSGSLSGSTNTLTITGVQVEDEAVYYC-G---D-SIDW---FGAGTTLTVL			

Fig. S3. SDS-PAGE analysis of anti-Thi scFv, scFv-biotin, and scFv-AP fusion protein.

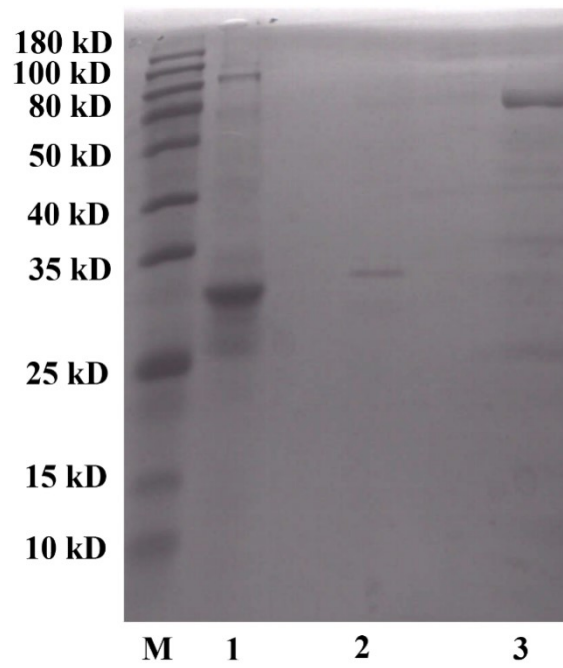


Fig. S4. Matrix effects of extracts diluted with PBS (pH 7.4) containing 5% MeOH on Thi2-7 scFv-biotin ELISAs. A) Apple, B) Pear, C) Cabbage, and D) Cucumber.

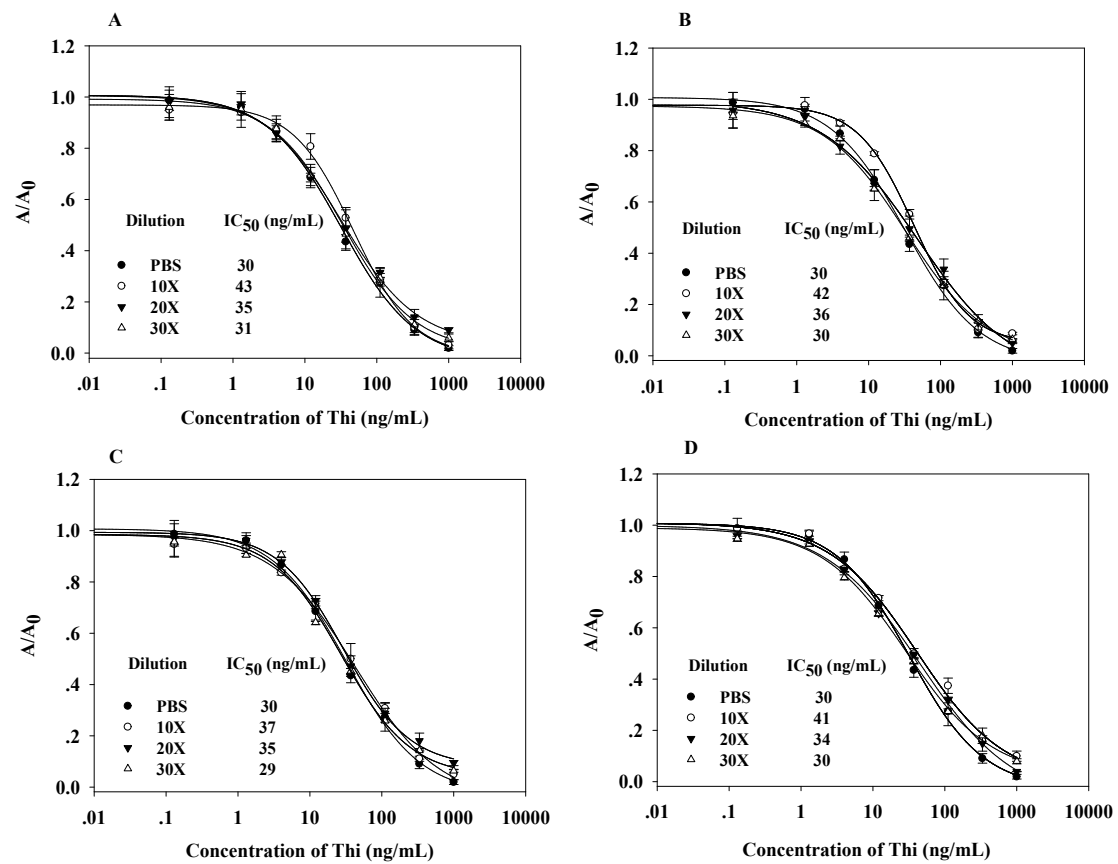


Fig. S5. Correlations between the Thi2-7 scFv-biotin based ELISA and the HPLC for Thi spiked in apple, pear, cabbage, and cucumber samples.

