Electronic Supplementary Material (ESI) for Analytical Methods.

IVT cell-free biosensors for tetracyclines and macrolides detection based on allosteric transcription factors (aTFs)

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Table S1. DNA Sequences

Label	Sequence	Purpose
F	gcggataacaatttcacacaggaaacagc	DNA template amplification forward primer
R	caaaaaaccccctcaagacccg	DNA template amplification reverse primer
Biotin-F	Biotin-gcggataacaatttcacacaggaaacagc	DNA template amplification forward primer for BIAcore /EMSA
Biotin-R	Biotin-caaaaaaccccctcaagacccg	DNA template amplification reverse primer for EMSA/BIAcore
Sense primers	ttaagattatgctgagtgatatccccccacatacacatggcaaga	For NASBA
Antisense primers	cccacatactctgatgatcc	For NASBA
T7-tetO-3WJdB-T	gcggataacaatttcacacaggaaacagctatgaccatgattacgccaagcttgcatgcctgcaggtcgactctagataatacgactcactataggaggtccctatcagtgatagagacccacatactctgatgatccgagacggtcgggtccagatattcgtatctgtcgagtagagtgtgggctcggatcattcatggcaagagacggtcgggtccagatattcgtatctgtcgagtagagtgtgggctcttgccatgt	For tetracyclines detection

T7-mphO-3WJdB-T	gtatgtgggtagcataacccctggggcctctaaacgggtcttgaggggttttttgcggataacaatttcacacaggaaacagctatgaccatgattacgccaagcttgcatgcctgcaggtcgactctagataatacgactcactataggagggaatataaccgacgtgactgttacatttaggtggcccacatactctgatgatccgagacggtcgggtccagatattcgtatctgtcgagtagagtgtgggctcggatcattcatggcaagagacggtcgggtccagatattcgtatctgtcgagtagagtgtgggctcttgccatgtgtatgtgggtagcataacccctggggcctctaaacgggtcttgaggggttttttg	For macrolides detection

Table S2. Preparation of electrophoresis gel of EMSA

Reagent	Stock Concentration	Volume
TBE Buffer (10×)	45mM	1mL
30% Acr-Bis	0.5μM	2.2 mL
Glycerol	80% (v/w)	80μL
Ammonium persulfate	10% (v/w)	90μL
TEMED	100 mM	10μL
ddH_2O		6.62mL

Table S3. Chemical components and concentration of NASBA

Reagent	Stock Concentration	Volume
IVT reaction solution	-	20 μL
sense and antisense primers	10 μM	0.4 μL
RNase inhibit	40 (U/μl)	0.5 μL
NTPs	100 mM	Each 0.4 μL
d NTPs Mix	10mM each	2 μL
65 ℃, 5 min, after 37 ℃, 5 min		
AMV	10 U/μL	1 μL
RNase H	5 U/μL	0.1 μL
T7 RNAP	200 U/μL	0.25 μL

Table S4. Consistency and stability of the aTFs-based biosensors. (n=3)

Sample	Spiked (μM)	Batche 1	Batche 2	Batche 3	Batche 4	Batche 5	RSD (%)
Anhydrot	1	0.93±0.05	1.04±0.08	0.90±0.03	0.95±0. 09	0.95±0.09	5.60
etracyclin	5	4. 61±0.31	4.51±0.28	4.16±0.39	4.37±0.17	4.39±0.22	3.84
e in milk	10	8.10±0.81	8.80±0.92	9.17±0.91	8.71±0.71	8.85±0.93	4.46
Erythrom	1	0.92±0.05	0.84±0.06	0.86±0.08	0.86±0.06	0.82±0.06	4.22
ycin in	5	4.93±0.17	4.47±0.17	4.37±0.51	4.15±0.15	4.32±0.47	6.58
milk	10	10.07±0.20	8.58±0.49	8.89±0.49	8.85±0.36	8.76±0.20	6.58

*RSD (%) means relative standard deviation between 5 batches .

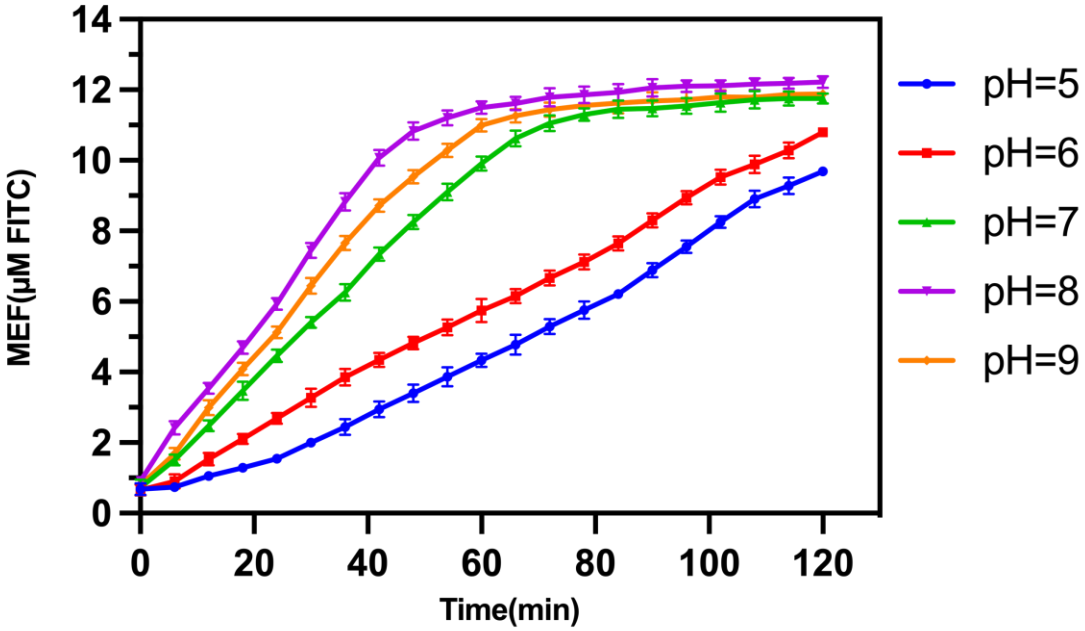

Figure S1. Effect of pH on IVT cell-free biosensor. MEF of biosensor at 120 min under different pH of IVT without aTFs. Error bars are means and SDs from three independent repeats.

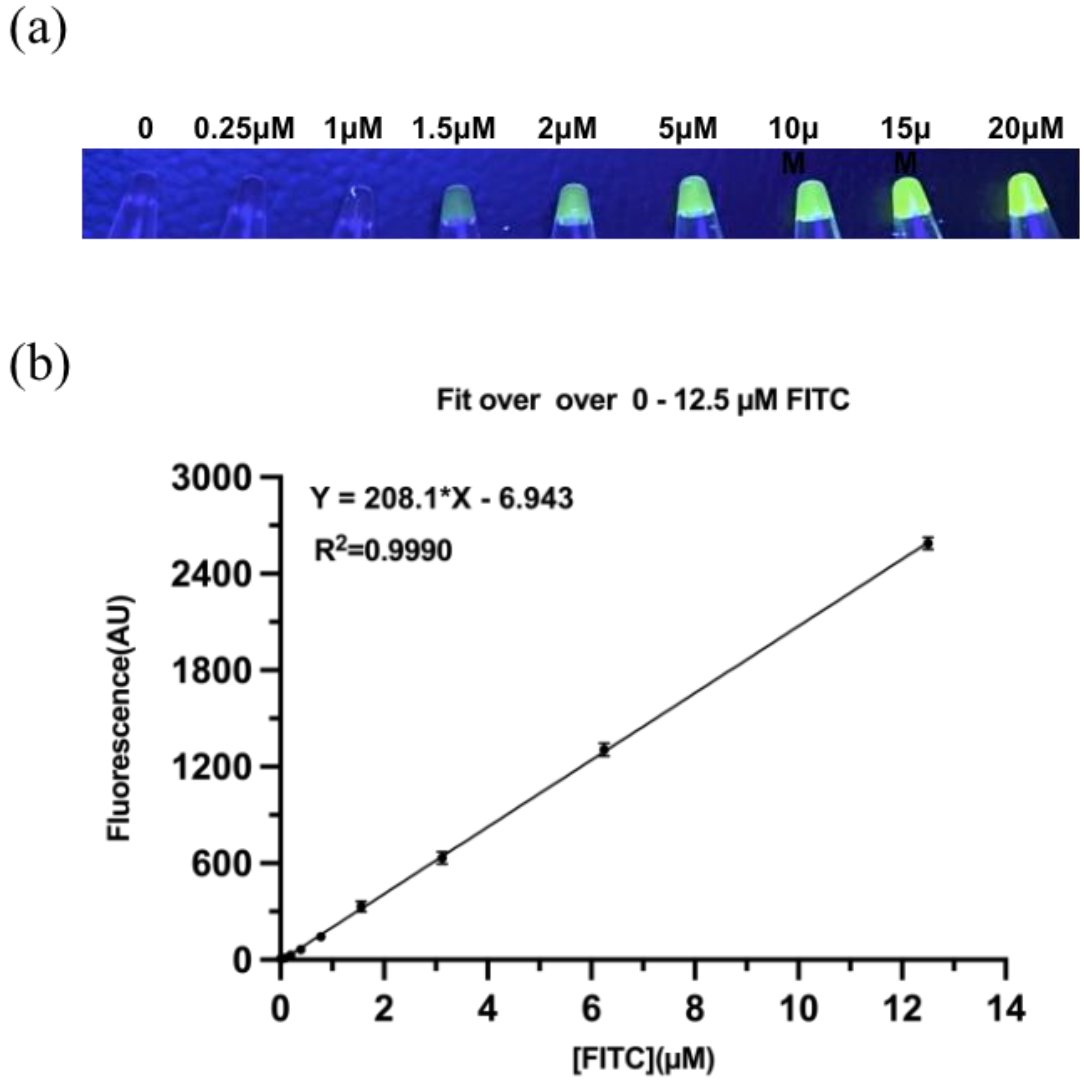

Figure S2. Arbitrary fluorescence values were converted to micromolar equivalent fluorescein (MEF). Fluorescence of different concentrations of FITC was measured at excitation and emission wavelengths of 472 and 507 nm by the plate reader. Error bars are SDs from nine independent repeats.