



Figure S1 Comparison of the results using DAB and AEC as chromogenic substrate.

Left: with AEC; Right: with DAB.

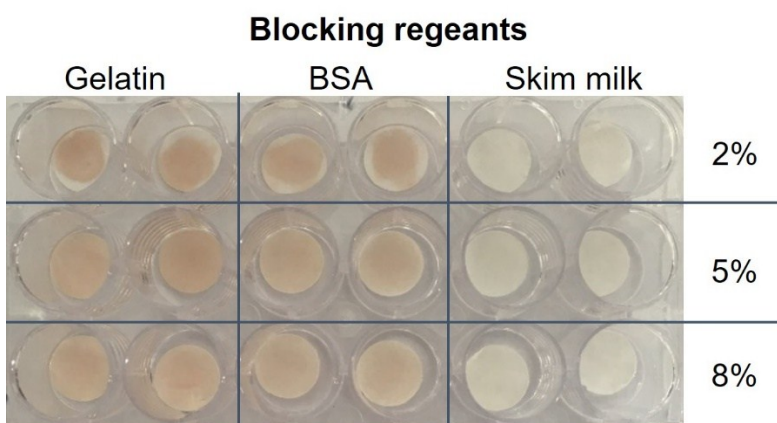


Figure S2. Optimization of blocking agents.

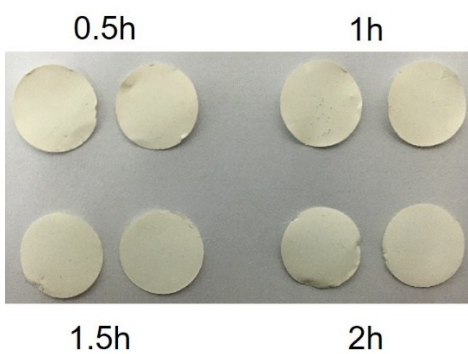


Figure S3. Optimization of blocking time.

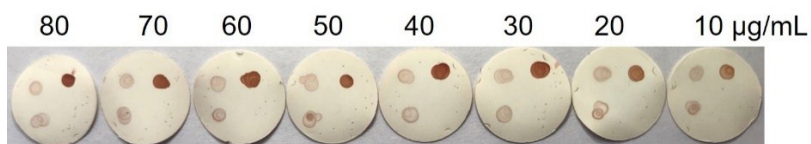











Figure S4. Optimization of goat anti-mouse antibody as the positive control.

Table S1 Cross-reactivity of mAb-KAN with analogs

Antibiotics	IC ₅₀ (ng/mL)	Cross-reactivity (%)
Kanamycin	2.15	100%
Tobramycin	2.17	99.07%
Amikacin	978.70	0.22
Streptomycin	13285.81	0.02
Dihydrostreptomycin	>20,000	<0.01
Neomycin	>20,000	<0.01
Gentamicin	>20,000	<0.01

Table S2. Comparison of Dual Dot-ELISA and UPLC-MS/MS Using Random Spiked

Samples

Spiked samples		Dual Dot-ELISA		UPLC-MS/MS	
		KAN (ng/mL)	STR (ng/mL)	KAN (ng/mL)	STR (ng/mL)
1		30.25 ± 2.21	55.32 ± 4.18	38.41 ± 3.23	42.72 ± 5.52
2		35.23 ± 2.35	4.66 ± 0.21	26.93 ± 2.32	6.61 ± 0.53
3		0.52 ± 0.03	4.14 ± 0.14	ND	6.35 ± 0.32
4		1.52 ± 0.11	15.34 ± 1.63	3.62 ± 0.25	10.32 ± 1.12
5		44.62 ± 7.35	11.35 ± 1.19	56.13 ± 7.28	16.63 ± 1.54
6		4.73 ± 0.32	46.14 ± 0.35	6.64 ± 0.54	53.42 ± 4.89
7		0.31 ± 0.01	55.32 ± 0.92	ND	62.17 ± 5.21
8		41.62 ± 0.38	ND	48.92 ± 5.68	ND
9		1.31 ± 0.13	2.31 ± 0.56	2.67 ± 0.18	5.31 ± 0.31

ND: not detected.