

2 **A Class-Selective Immunoassay for Sulfonamides**  
3 **Residue Detection in Milk using a Superior**  
4 **Polyclonal Antibody with Broad Specificity and**  
5 **Highly Uniform Affinity**

6 **Chenglong Li**<sup>1</sup>, **Xiangshu Luo**<sup>1</sup>, **Yonghan Li**<sup>2</sup>, **Huijuan Yang**<sup>1</sup>, **Xiao Liang**<sup>3</sup>, **Kai Wen**<sup>1</sup>,  
7 **Yanxin Cao**<sup>1</sup>, **Chao Li**<sup>1</sup>, **Weiyu Wang**<sup>1</sup>, **Weimin Shi**<sup>1</sup>, **Suxia Zhang**<sup>1</sup>, **Xuezhi Yu**<sup>1,\*</sup> and  
8 **Zhanhui Wang**<sup>1</sup>

9 <sup>1</sup> Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Veterinary  
10 Medicine, China Agricultural University, Beijing Key Laboratory of Detection Technology for  
11 Animal-Derived Food Safety, Beijing Laboratory of Food Quality and Safety, 100193 Beijing,  
12 People's Republic of China

13 <sup>2</sup> Henan Animal Health Supervision Institute, 450008 Zhengzhou, People's Republic of China

14 <sup>3</sup> College of Veterinary Medicine, Qingdao Agricultural University, 266109 Qingdao, People's  
15 Republic of China

16 \* Correspondence: yu51422396@126.com; Tel.: +86-10-6273 4565; Fax: +86-10-6273 1032

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18 **Figure Captions**

19 **Figure S1.**  $^1\text{H}$ -NMR result of hapten SA10-X

20 **Figure S2.**  $^{13}\text{C}$ -NMR result of hapten SA10-X

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**Table S1.**

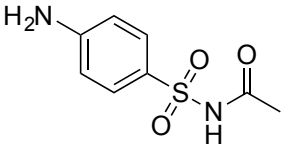
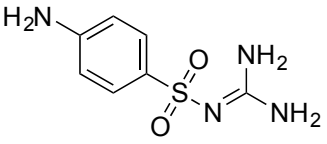
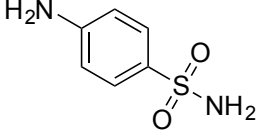
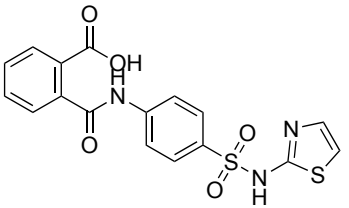
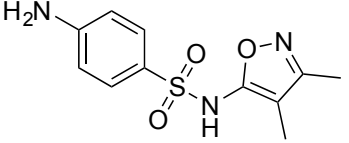
Coating antigens selecting result of pAb

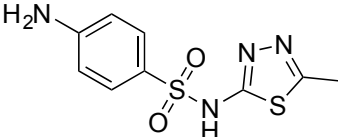
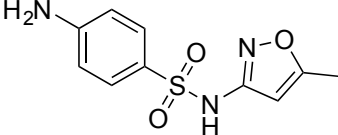
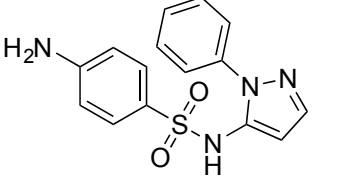
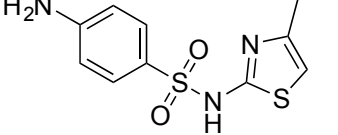
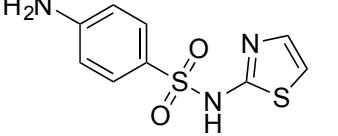
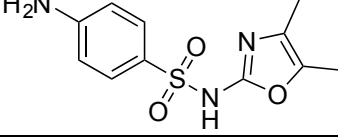
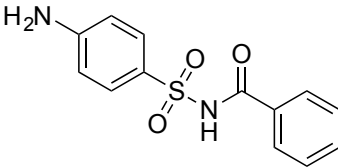
Coating antigens	Coating antigens dilution	pAb dilution	B <sub>0</sub>	B (100 µg L <sup>-1</sup> )	IC <sub>50</sub> of SMZ (µg L <sup>-1</sup> )
BS-OVA	1:160,000	1:100,000	1.950	1.067	65.5
TS-OVA	1:5,000	1:20,000	1.749	1.188	174.3
ST-OVA	1:20,000	1:40,000	1.304	0.886	235.8
SA10-X-OVA	1:500,000	1:200,000	1.596	1.538	>500
SMZ-OVA	1:10,000	1:10,000	0.245	NA	NA
SG-OVA	1:10,000	1:10,000	0.235	NA	NA
HS-OVA	1:10,000	1:10,000	0.150	NA	NA
SA10-OVA	1:10,000	1:10,000	0.134	NA	NA

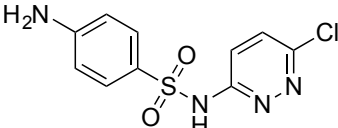
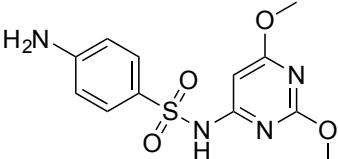
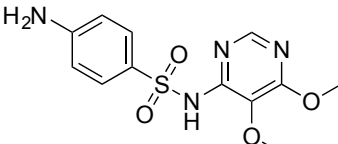
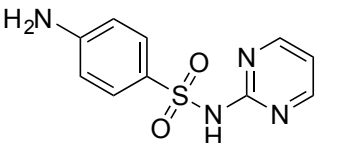
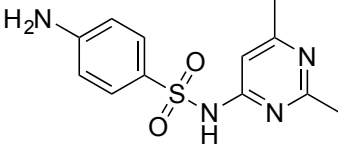
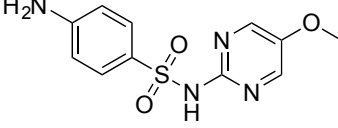
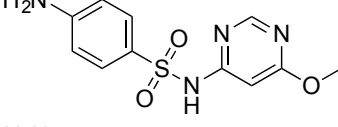
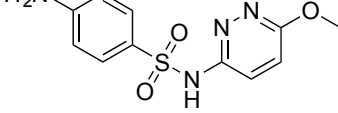
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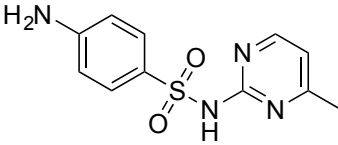
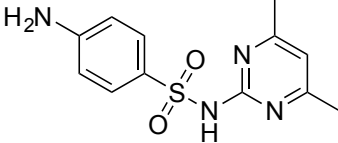
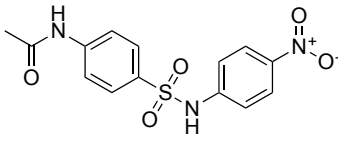
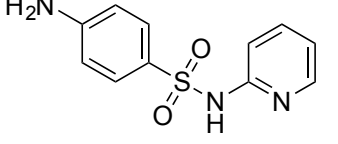
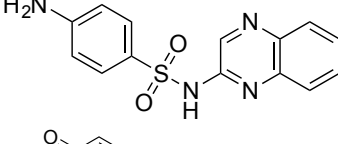
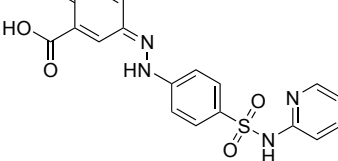
**Table S2.**

IC<sub>50</sub> values (μg L<sup>-1</sup>) of SAs against pAb in this study and other antibodies in literatures

SAs	Structures	pAb	pAb HS [1]	pAb SAB [2]	pAb SA1-CH <sub>2</sub> [3]	27G3 [4]	pAb SA2' [3]	pAb D3 [5]	Sulfa-1 [6]	4E10 [4]	3B5B 10E3 [7]	4E5 [8]	rAb [9]	Chen [10]
<b>Single-ring SAs</b>														
SA		10,000	10,000	ND	2367	1800	452	ND	14,349	10,000	ND	100	7.9	41.16
SG		127.74	8614	63	1201	500	731	ND	ND	10,000	ND	100	12	30.24
SN		2122.4	7524	108	43.5	ND	19.0	ND	60,000	ND	ND	100	1800	90.18
<b>Two-ring SAs containing a five-atom ring</b>														
PST		85.69	6531	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	60.16
SIZ		164.53	10,000	ND	10,000	250	95.6	200	200,000	80	350	51	0.51	35.62

SMT		3.56	113.4	399	70.3	0.6	8.8	12.8	70,000	2	ND	39	0.019	0.08
SMX		10.31	305.4	88	255	150	0.4	135	60,000	30	9	25	0.78	0.15
SPA		15.01	3024	ND	3098	ND	49.5	ND	ND	ND	ND	ND	0.39	28.27
ST		3.71	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
STZ		37.7	241.1	24	12.5	10	13.7	2	322	130	30	3.2	0.21	0.39
SXL		25.51	1590	ND	ND	ND	ND	150	ND	ND	ND	ND	ND	29.91
<b>Two-ring SAs containing a six-atom ring</b>														
SBA		2349	111.8	ND	ND	ND	ND	ND	100,000	ND	ND	ND	ND	26.69

SCP		9.75	786.9	23	3.8	4	10.7	14	4360	4	20	7.3	0.072	0.39
SDM		10.34	345.8	137	171	250	6.1	190	15,724	100	100	5.3	9.9	3.45
SDM'		8786.2	5105	ND	1731	30	426	31	ND	800	ND	0.52	0.32	ND
SDZ		37.88	1282	36	18.2	80	19.5	32	9763	80	30	0.92	0.51	4.89
SIM		6.49	1302	ND	ND	ND	ND	1.3	3136	ND	ND	1.9	ND	48.39
SMD		1.47	534.3	ND	6.7	ND	2.7	3.2	ND	ND	ND	0.76	ND	0.81
SMM		5.12	512.4	ND	ND	ND	ND	ND	ND	ND	ND	1.1	0.62	4.51
SMP		0.92	119.5	1.6	0.6	15	0.6	8.4	ND	13	ND	7.4	0.18	0.57

SMR		11.5	1419	18.8	16.1	500	36.2	19	3743	250	600	1.4	0.65	1.53
SMZ		50.78	8337	118	582	8000	311	130	70,000	7000	1050	5.8	13	18.79
SNT		119.9	47.2	ND	ND	ND	ND	ND	1.41	ND	ND	ND	10	37.16
SPY		23.68	124.4	ND	0.7	30	0.4	10	22.8	150	350	100	0.26	5.09
SQX		12.18	1053	455	4	1200	1.4	90	25,000	3000	ND	3.8	7.1	39.12
SSA		15.16	ND	ND	ND	ND	ND	ND	4305	ND	ND	ND	1100	80.05

ND, not detected; rAb, recombinant antibody

**Table S3.**

Optimized parameters of indirect competitive ELISA based on pAb using SMZ as reference analyte

Factors	Conditions	B <sub>0</sub>	IC <sub>50</sub> ( $\mu\text{g L}^{-1}$ )	B <sub>0</sub> /IC <sub>50</sub>	Factors	Conditions	B <sub>0</sub>	IC <sub>50</sub> ( $\mu\text{g L}^{-1}$ )	B <sub>0</sub> /IC <sub>50</sub>
Coating solutions	<b>CB</b>	<b>1.342</b>	<b>60.3</b>	<b>0.0223</b>	NaCl (M)	0	1.473	68.8	0.0214
	PBS	1.275	62.8	0.0203		<b>0.05</b>	<b>1.337</b>	<b>50.3</b>	<b>0.0266</b>
	BB	0.2	NA			0.15	1.246	54.5	0.0229
Coating condition	37°C 2 h	1.342	60.3	0.0223	0.3	1.087	57.2	0.0190	
	<b>4°C overnight</b>	<b>1.357</b>	<b>55.5</b>	<b>0.0245</b>	0.5	0.875	61.7	0.0142	
	37°C 2 h+4°C overnight	1.477	57.2	0.0258					
Blocking buffer	<b>5% FBS</b>	<b>1.349</b>	<b>51.8</b>	<b>0.0260</b>	Tween-20 (%)	<b>0</b>	<b>1.373</b>	<b>51.8</b>	<b>0.0265</b>
	0.25% casein	1.009	52.6	0.0192		0.05	1.634	63.3	0.0258
	5% skimmed milk	0.896	54.9	0.0163		0.1	1.845	74.5	0.0248
						0.5	1.833	97.2	0.0189
				2	1.921	101.5	0.0189		
Blocking time	30 min	1.527	78.9	0.0194	Competitive time	15min	0.816	62.5	0.0131
	1 h	1.435	57.3	0.0250		<b>30 min</b>	<b>1.357</b>	<b>50.4</b>	<b>0.0269</b>
	<b>2 h</b>	<b>1.314</b>	<b>53.2</b>	<b>0.0247</b>		45 min	1.364	53.2	0.0256
	4 h	1.266	53.5	0.0237		60 min	1.387	63.3	0.0219
pH	5.0	1.079	75.3	0.0143	Reaction time of IgG-HRP	15 min	0.735	57.2	0.0128
	6.0	1.363	63.5	0.0215		<b>30 min</b>	<b>1.349</b>	<b>50.8</b>	<b>0.0266</b>
	<b>7.4</b>	<b>1.374</b>	<b>54.2</b>	<b>0.0254</b>		45 min	1.577	53.7	0.0294
	8.0	1.382	67.7	0.0204		60 min	1.723	55.4	0.0311
	9.0	0.977	89.2	0.0110					



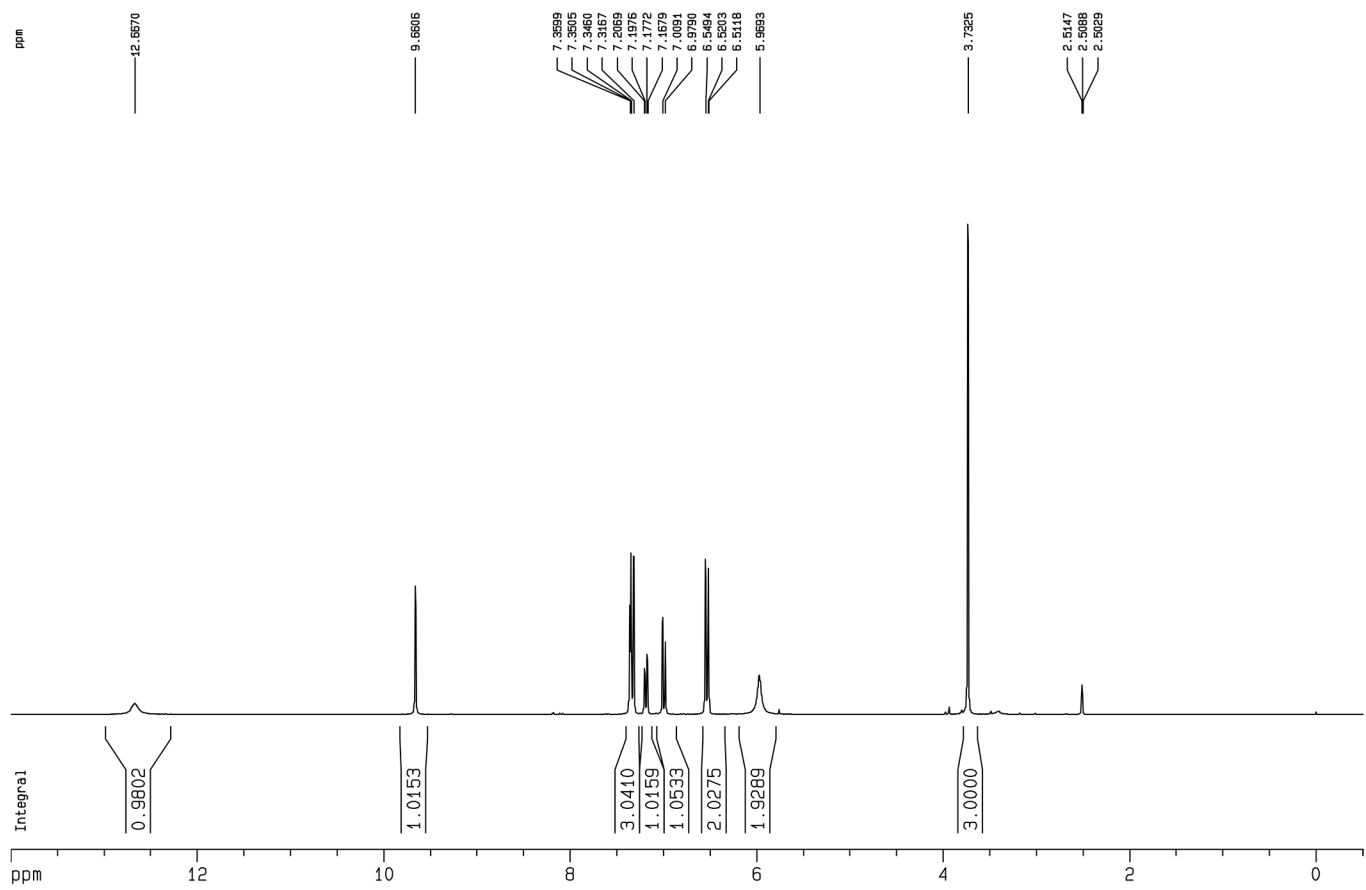


Figure S1.

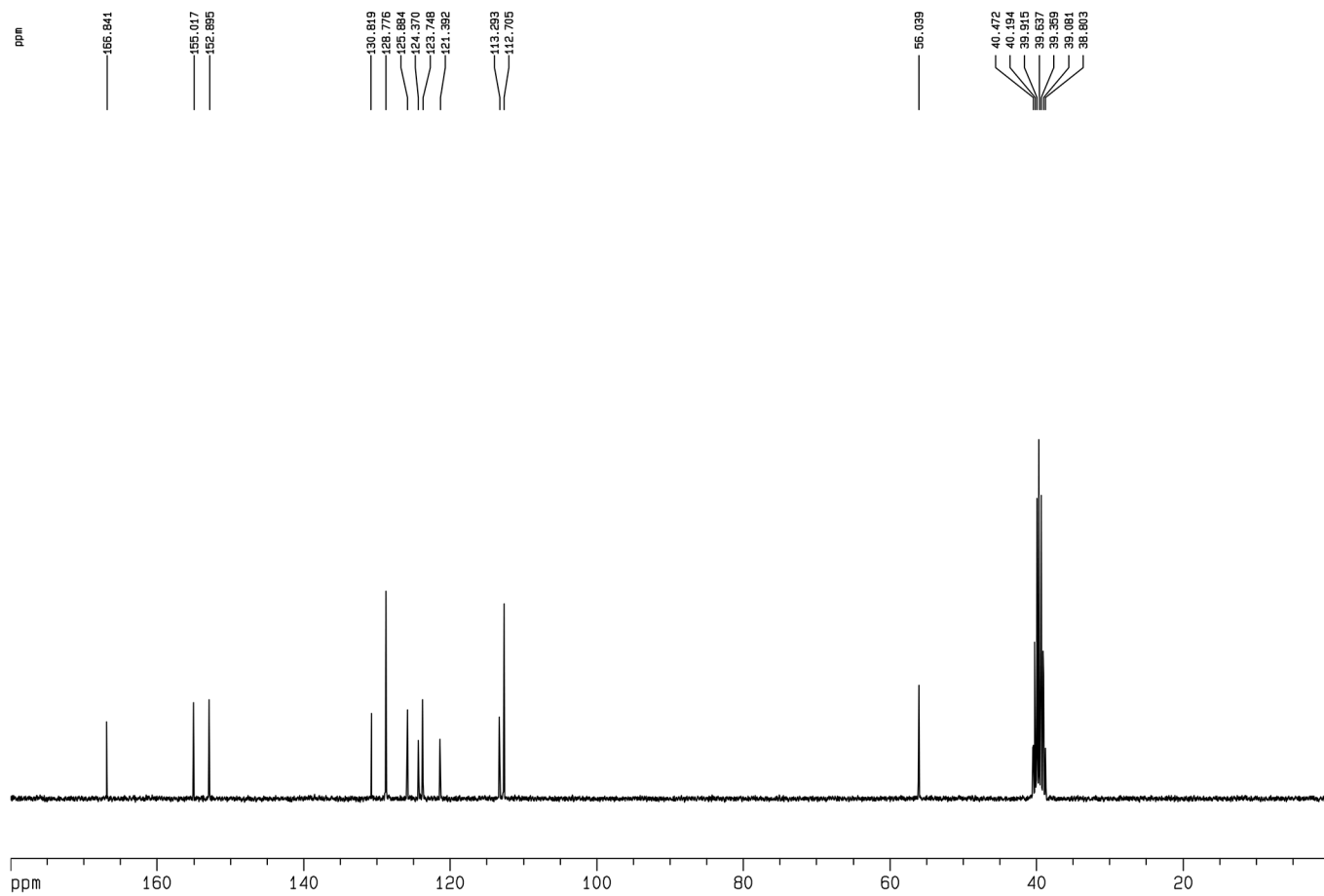


Figure S2.

## References

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