

Supplementary Material

Immunogenic Response for the Production of Polyclonal Antibodies of Human Serum Amyloid A1 (hSAA1) in Rabbits and Mice

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Supplementary Table I: Posphate-buffered saline (PBS) 1 liter according to cold spring Harbor Laboratory*.

Reagent	For 1X working solution	Final concentration 1X working solution	For 10X working solution	Final concentration 10X working solution
NaCl	8 g	137 mM	80 g	1.37 M
KCl	0.2 g	2.7 mM	2 g	27 mM
Na ₂ HPO ₄	1.44 g	10 mM	14.4 g	100 mM
KH ₂ PO ₄	0.24 g	1.8 mM	2.4 g	18 mM

* <https://cshprotocols.cshlp.org/>

Supplementary Table II: Comparison of titer analysis of hSAA1 polyclonal antibodies raised in rabbit and mice using fixed amount of hSAA1 antigen (200 ng).

Mice antisera dilution	Titer analysis by OD ₄₅₀ nm	Rabbit antisera dilution
100-5000	>2.0	100-10,000
10,000-60,000	2-1.0	20,000-60,000
80,000-100,000	1.0-0.5	80,000-200,000
150,000-500,000	<0.5	250,000-500,000

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0030-9923/2025/0006-2767 \$ 9.00/00

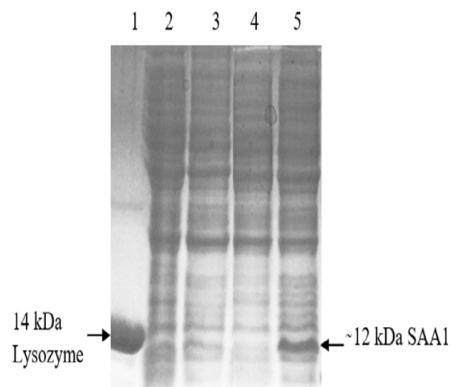


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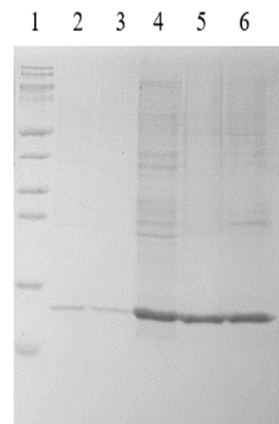
		M	K	L	L	T	G	L	V	F	C	S	L	V	L	G	V	S	S	R	S	
Native	CAT	ATG	AAG	CTT	CTC	ACG	GGC	CTG	GTT	TTC	TGC	TCC	TTG	GTC	CTG	GGT	GTC	AGC	AGC	CGA	AGC	63
Synthetic	---	ATG	AAA	CTG	CTG	ACC	GGT	CTG	GTT	TTC	TGC	TCT	CTG	GTT	CTG	GGT	GTT	TCT	TCT	CGT	TCT	63
		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	
	F	E	S	F	L	G	E	A	F	D	G	A	R	D	M	W	R	A	Y	S		
Native	TTC	TTT	TCG	TTC	CTT	GGC	GAG	GCT	TTT	GAT	GGG	GCT	CGG	GAC	ATG	TGG	AGA	GCC	TAC	TCT	123	
Synthetic	TTC	TTT	TCT	TTC	CTG	GGT	GAA	GCA	TTC	GAC	GGT	GCG	CGT	GAC	ATG	TGG	CGT	GCT	TAC	TCT	123	
	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	*	***	***	***	
	D	M	R	E	A	N	Y	I	G	S	D	K	Y	F	H	A	R	G	N	Y		
Native	GAC	ATG	AGA	GAA	GCC	AAT	TAC	ATC	GGC	TCA	GAC	AAA	TAC	TTC	CAT	GCT	CGG	GGG	AAC	TAT	183	
Synthetic	GAT	ATG	CGT	GAA	GCT	AAC	TAC	ATT	GGT	TCT	GAT	AAA	TAC	TTC	CAC	GCT	CGT	GGT	AAC	TAT	183	
	***	***	*	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	
	D	A	A	K	R	G	P	G	G	V	W	A	A	E	A	I	S	D	A	R		
Native	GAT	GCT	GCC	AAA	AGG	GGA	CCT	GGG	GGT	GCC	TGG	GCT	GCA	GAA	GTG	ATC	ACC	GAT	GCC	AGA	243	
Synthetic	GAC	GCT	GCT	AAA	CGT	GGT	CCG	GGT	GGT	GTT	TGG	GCT	GCT	GAA	GCT	ATC	TCT	GAT	GCT	CGT	243	
	***	***	***	***	***	***	***	***	***	*	***	***	***	***	***	***	***	***	***	*		
	E	N	I	Q	R	F	E	G	H	G	A	E	D	S	L	A	D	Q	A	A		
Native	GAG	AAT	ATC	CAG	AGA	TTC	TTT	GGC	CAT	GGT	GCG	GAG	GAC	TCG	CTG	GCT	GAT	CAG	GCT	GCC	303	
Synthetic	GAA	AAC	ATC	CAG	CGT	TTC	TTT	GGT	CAC	GGT	GCT	GAA	GAT	TCT	CTG	GCT	GAT	CAG	GCT	GGT	303	
	***	***	***	***	*	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	
	N	E	W	G	R	S	G	K	D	P	N	H	F	R	P	A	G	L	P	E		
Native	AAT	GAA	TGG	GGC	AGG	AGT	GGC	AAA	GAC	CCC	AAT	CAC	TTC	CGA	CCT	GCT	GGC	CTG	CCT	GAG	363	
Synthetic	AAC	GAA	TGG	GGT	CGT	TCT	GGT	AAA	GAT	CCG	AAC	CAC	TTC	CGT	CCG	GCT	GGT	CTG	CCG	GAA	363	
	***	***	***	***	*		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	
	K	Y																				
Native	AAA	TAC	TGA	---	---	378																
Synthetic	AAA	TAC	TAA	AAG	CTT	378																
	***	***	*	*	*																	

Supplementary Fig. 1. Sequence alignment of synthetic human *SAA1* gene having *Escherichia coli* preferred codons with native *SAA1*. The highlighted sequences show alteration in genetic codon. Emboldened and underlined sequences show signal peptide.



Supplementary Fig. 2. 15% Tricine PAGE for expression

analysis of recombinant human SAA1
 Lane 1: Lysozyme as a protein marker
 Lane 2: Uninduced pET-21a vector
 Lane 3: pET-21a vector induced with 0.2mM IPTG
 Lane 4: Uninduced *hSAA1-pET21a*
 Lane 5: *hSAA1-pET21a* induced with 0.2mM IPTG



Supplementary Fig. 3. 15 % Tricine-PAGE of purified recombinant human SAA1

Lane 1: Protein marker (26616) PageRuler™
 Lane 2: Reductive sample of combined fraction of Octyl-FF (reverse phase hydrophobic column)
 Lane 3: Non-reductive sample of combined fraction of Octyl-FF
 Lane 4: Dialysed SAA1 protein sample before purification
 Lane 5: Non-reductive sample of combined fraction of Q-FF (anion exchange column)
 Lane 6: Reductive sample of combined fraction of Q-FF