

CERTIFICATE OF ANALYSIS

Product: Polyclonal anti-S-Adenosylmethionine (SAM) antibody R3

Catalog Number: PA00201

Lot Number: Lot#PP420825, Lot# MP422421

Document Release Date: March 18, 2015

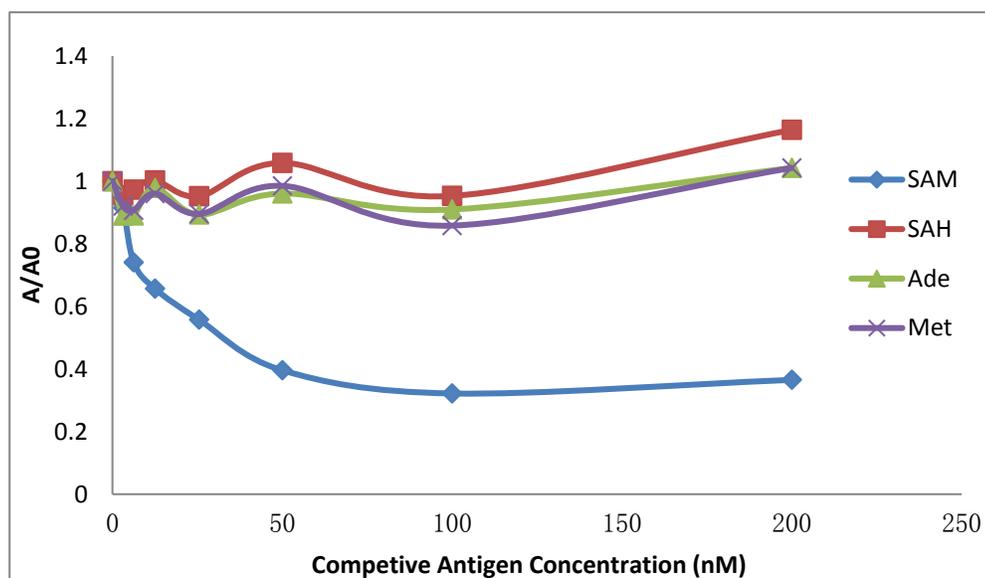
Test	Specification	Result
Appearance	Clear liquid	Clear solution
Concentration (by UV Spectrophotometer)	About 1 mg/ml	About 1mg/ml
Storage buffer	10mM PBS pH 7.4 (150 mM NaCl) , 10mg/ml BSA,0.02%NaN3, 50%Glycerol	10mM PBS pH 7.4 (150 mM NaCl) , 10mg/ml BSA,0.02%NaN3, 50%Glycerol
Stability at 37°C	Few hours	Same dilution in 14 days
Stability at 4°C	30 days	Same dilution in 48 days
Stability at -20°C	>1 year	>1 year
Stability at freeze and thaw	Avoid repeated freeze-and-thaw	No change in antigen-binding capability after freezer and thaw 14 times
Stability with dilution up to 5 folds	Diluted antibodies may be less stable	No difference during the period of thermal stability tests above
Specificity	Cross reaction with analogs <1%	Cross reaction with analogs <1%
Sensitivity (by cELISA)	PBS: 12.5 - 50nM	PBS: 12.5 - 50nM
Affinity (by cELISA)	$K_a \geq 10^8$ L/mol	$K_a = 9.58 \times 10^8$ L/mol = 1.04 nM

Specificity

(1) Dosage-dependent competition was detected as a sample was added to a cELISA (Any SAM from a sample competes with the coated SAM heptan to bind anti-SAM polyclonal antibody R3). The sample is the product of the following biochemical reaction: Methionine Adenosyltransferase (MAT) was added to methionine and adenosine triphosphate under an appropriate buffer at 37°C. It indicates that antibody R3 specifically binds physiologically produced SAM.

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(2) Cross reaction Figure below showed a competitive ELISA with anti-S-Adenosymethionine polyclonal antibody [R3] (PA00201). The 0.1 µg/ml of SAM coating standard (Cat # ACT00201) was coated into 96 wells. Serial dilution of SAM standard (Cat # AST00201), S-Adenosylhomocysteine (SAH), Adenosine (Ade), L-Methionine (Met) and 1:15000 of rabbit anti-SAM serum was added. HRP conjugated Goat anti-Rabbit IgG antibody was used to develop the color. The A is the OD450 value of the test well and the A0 is the OD450 of the well without competitive antigen.



Sensitivity

Table 1 showed indirect cELISA result with 0.25 µg/ml PLL-aza-SAM coated micro-titer plate, Rabbit polyclonal anti-SAM dilution of 1:2,000 in PBS incubation system. Different amounts of coating antigen, antibody, and incubating buffer will give slightly different minimum detection limits.

Table 1 Standard curve in indirect cELISA in PBS

Standards (nM)	OD
0	1.1011
12.5	0.9393
25	0.9188
50	0.7021
100	0.8002

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200	0.6978
400	0.4540
Blank	0.0116

Affinity

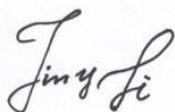
Table 2 Anti-SAM Polyclonal Antibodies Binding to Different Amount of Antigen

Dilution from 1mg/ml	Polyclonal anti-SAM R3 PLL-aza-SAM	
	0.05µg/ml	0.1µg/ml
25	4.9225	4.9514
50	4.8524	4.9929
100	4.3849	5.1149
200	3.8518	4.8695
400	3.0971	4.1802
800	2.2404	3.3979
1600	1.5083	2.4903
3200	1.0336	2.0391

From Table 2 when coating antigen was at 0.1µg/ml, half the maximum OD was seen at about 1:1500. The corresponding antibody concentration was $[Ab] = (1\text{mg/ml}/160,000\text{g/mol})/1500 = 4.167 \times 10^{-9}$. When coating antigen was at 0.05µg/ml, half the maximum OD was seen when R3 was diluted at about 1:800. The corresponding antibody concentration was $[Ab]_t = (1\text{mg/ml}/160,000\text{g/mol})/800 = 7.812 \times 10^{-9}$.

$$n = (0.1\mu\text{g/ml})/(0.05\mu\text{g/ml}) = 2$$

$$K_a = (n-1)/2 * (n[Ab] - [Ab]_t) = 9.58 \times 10^8 \text{ L/mol} = 1.04 \text{ nM}$$



Jimmy Li
 Quality Control Team

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