

CERTIFICATE OF ANALYSIS

Product: Monoclonal anti-S-Adenosylmethionine (SAM) antibody clone 84-3

Catalog Number: MA00202

Lot Number: Lot#MP420825, Lot#MP421816, Lot#MP421212, Lot#MP421815, Lot# MP422421
 Lot# MP451607, Lot# MP480213

Document Release Date: July 24, 2017

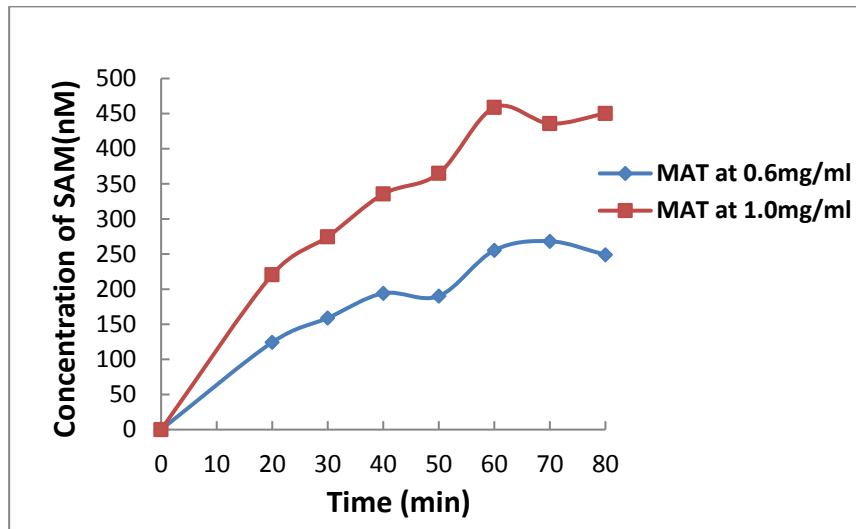
Test	Specification	Result
Appearance	Clear liquid	Clear solution
Concentration (by UV Spectrophotometer)	About 0.5 - 1 mg/ml	About 0.5 - 1mg/ml
Storage buffer	10mM PBS pH 7.4 (150 mM NaCl) , 10mg/ml BSA,0.02%NaN3, 50%Glycerol	10mM PBS pH 7.4 (150 mM NaCl) , 10mg/ml BSA,0.02%NaN3, 50%Glycerol
Stability 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: 2nM Plasma: 30 - 60nM	PBS: 2nM Plasma: 30-60nM
Affinity (by cELISA, see details below)	$K_a \geq 10^9$ L/mol	$K_a = 7.29 \times 10^{10}$ L/mol $= 1.37 \times 10^{-11}$ M

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 hapten to bind HRP-conjugated antibody 84-3). The sample is the product of the following biochemical reaction: Methionine Adenosyltransferase (MAT) was added to

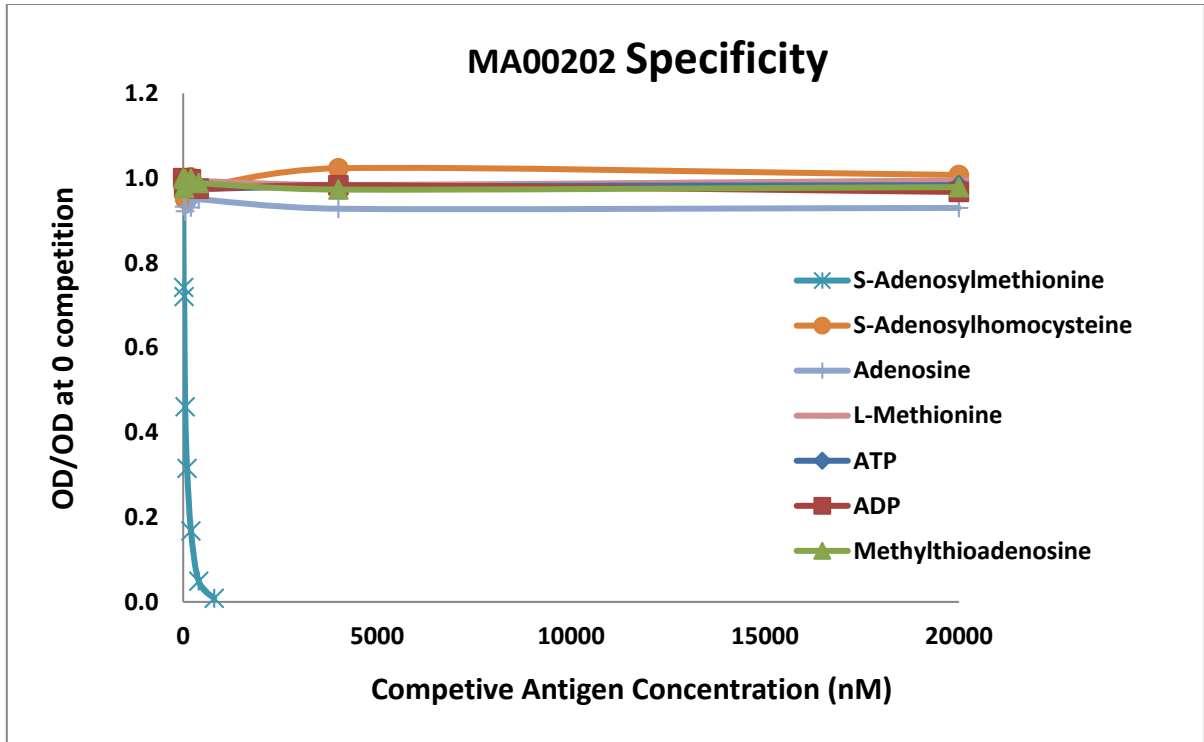
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methionine and adenosine triphosphate under an appropriate buffer at 37°C. It indicates that antibody 84-3 specifically binds physiologically produced SAM.



(2) Cross reaction Figure below showed a competitive ELISA with anti-S-Adenosymethionine monoclonal antibody [84-3] (MA00202). 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), Methythioadenosine (MTA), Adenosine diphosphate (ADP), Adenosine triphosphate (ATP) and 1:35000 of MA00202 were added. HRP conjugated Goat anti-Mouse IgG antibody was used to develop the color.

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Sensitivity

Different amounts of coating antigen, antibody, and incubating buffer will give slightly different minimum detection limits. Table 1 shows the standard curves measured from 3 different coating plates. ELISA was performed in triplicates. Mouse anti-SAM antibody 84-3 was used at 1:35000. The detecting sensitivity was estimated by the corresponding standard concentration calculated from standard curve equation based on the OD450 (OD450 at zero antigen – 2 x standard deviation of blank wells).

Table 1 OD450 measured in different amounts of coating antigens

SAM standard (nM)	PLL-aza-SAM								
	0.2µg/ml			0.15µg/ml			0.1µg/ml		
Blank	0.0498	0.056	0.0522	0.0476	0.0471	0.0482	0.0754	0.0515	0.0476
0	1.4709	1.4344	1.4072	1.215	1.2484	1.2651	1.0844	1.1231	1.0633

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6.25	1.365	1.3257	1.2673	1.0789	1.0734	1.0842	0.879	0.9122	0.8995
25	1.1769	1.1498	1.1015	0.8231	0.844	0.8334	0.6209	0.6561	0.6679
50	1.0230	0.9856	0.9577	0.6428	0.6362	0.6005	0.4517	0.4555	0.5187
100	0.7687	0.7532	0.7292	0.4292	0.4203	0.4427	0.3036	0.3103	0.3071
Sensitivity		3.0nM			1.7nM			1.6nM	

Affinity

Table 2 Anti-SAM Antibodies (84-3) Binding to Different Amount of Antigen

Dilution from 1mg/ml	Monoclonal anti-SAM 84-3 PLL-aza-SAM	
	0.05µg/ml	0.1µg/ml
1000	3.3764	4.7244
2000	3.2469	4.5207
4000	3.1591	4.5844
8000	2.9176	4.3432
16000	2.6605	4.2673
32000	2.1978	3.7642
64000	1.6954	3.1152
128000	1.2151	2.3332

From Table 2 when coating antigen was at 0.1µg/ml, half the maximum OD was seen at about 1:130000. The corresponding antibody concentration was $[Ab] = (1\text{mg/ml}/160,000\text{g/mol})/130000 = 4.807 \times 10^{-11}$ M. When coating antigen was at 0.05µg/ml, half the maximum OD was seen when 84-3 was diluted at about 1:70000. The corresponding antibody concentration was $[Ab]_t = (1\text{mg/ml}/160,000\text{g/mol})/70000 = 8.92 \times 10^{-11}$ M.

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

$$K_a = (n-1)/2*(n[Ab] - [Ab]_t) = 7.29 \times 10^{10} \text{ L/mol} = 1.37 \times 10^{-11} \text{ M}$$

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