

CERTIFICATE OF ANALYSIS

Product: Monoclonal anti-S-Adenosylmethionine (SAM) antibody clone 84-19

Catalog Number: MA00204

Lot Number: Lot#MP422429, Lot#MP421815, Lot#MP421816, Lot#MP420825

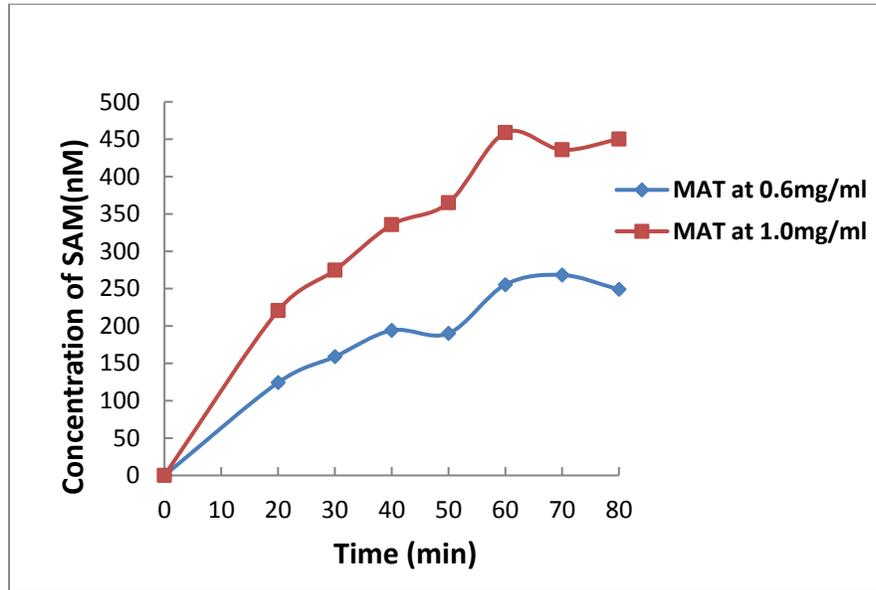
Document Release Date: July 24, 2017

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 4 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	$K_a = 7.75 \times 10^{10}$ L/mol $= 1.29 \times 10^{-11}$ M

Specificity

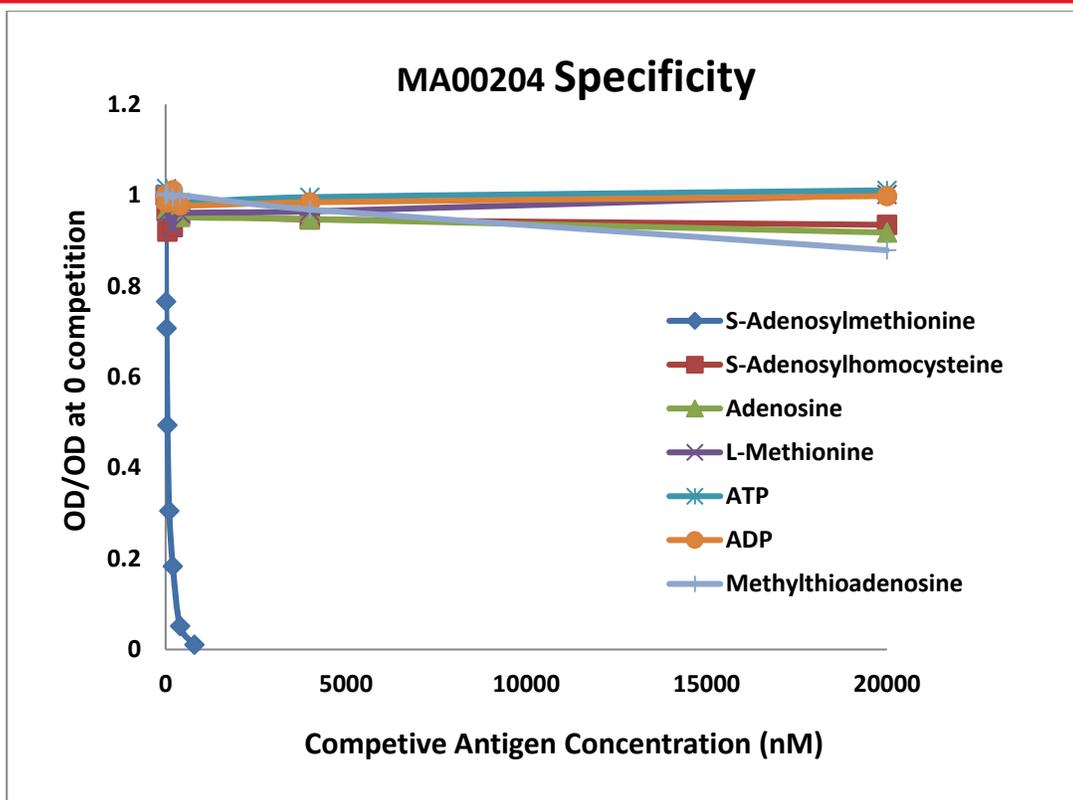
(1) Dosage-dependent competition was detected as a sample was added to a cELISA (Any SAM from a sample competes with the coated SAM heptan to bind HRP-conjugated antibody 84-19). The sample is the product of the following biochemical reaction: Methionine Adenosyltransferase (MAT) was added to methionine and adenosine triphosphate under an appropriate buffer at 37°C. It indicates that antibody 84-19 specifically binds physiologically produced SAM.

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(2) Cross reaction Figure below showed a competitive ELISA with anti-S-Adenosymethionine monoclonal antibody [84-19] (MA00204). The 0.1 $\mu\text{g}/\text{ml}$ 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 1:15000 of MA00204 were added. HRP conjugated Goat anti-Mouse IgG antibody was used to develop the color.

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Sensitivity

Similar to clone 84-3

Affinity

Table 1 Anti-SAM Antibodies (84-19) Binding to Different Amount of Antigen

Dilution from 1mg/ml	Monoclonal anti-SAM 84-19 PLL-aza-SAM	
	0.05µg/ml	0.1µg/ml
1000	3.1187	4.5631
2000	3.2047	4.5189
4000	3.1486	4.4623
8000	2.8566	4.3391
16000	2.5879	4.0764
32000	2.1472	3.5823

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64000	1.6419	2.8977
128000	1.1765	2.1967
256000	0.8865	1.8896

From Table 2 when coating antigen was at 0.1µg/ml, half the maximum OD was seen at about 1:120000. The corresponding antibody concentration was $[Ab] = (1\text{mg/ml}/160,000\text{g/mol})/120000 = 5.21 \times 10^{-11} \text{ M}$. When coating antigen was at 0.05µg/ml, half the maximum OD was seen when 84-19 was diluted at about 1:64000. The corresponding antibody concentration was $[Ab]_t = (1\text{mg/ml}/160,000\text{g/mol})/64000 = 9.765 \times 10^{-11} \text{ M}$.

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

$$K_a = (n-1)/2*(n[Ab] - [Ab]_t) = 7.75 \times 10^{10} \text{ L/mol} = 1.29 \times 10^{-11} \text{ M}$$

Jimmy Li
Quality Control Team

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