

CERTIFICATE OF ANALYSIS

Product: Monoclonal anti-S-Adenosylmethionine (SAM) antibody clone 118-18

Catalog Number: MA00203

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

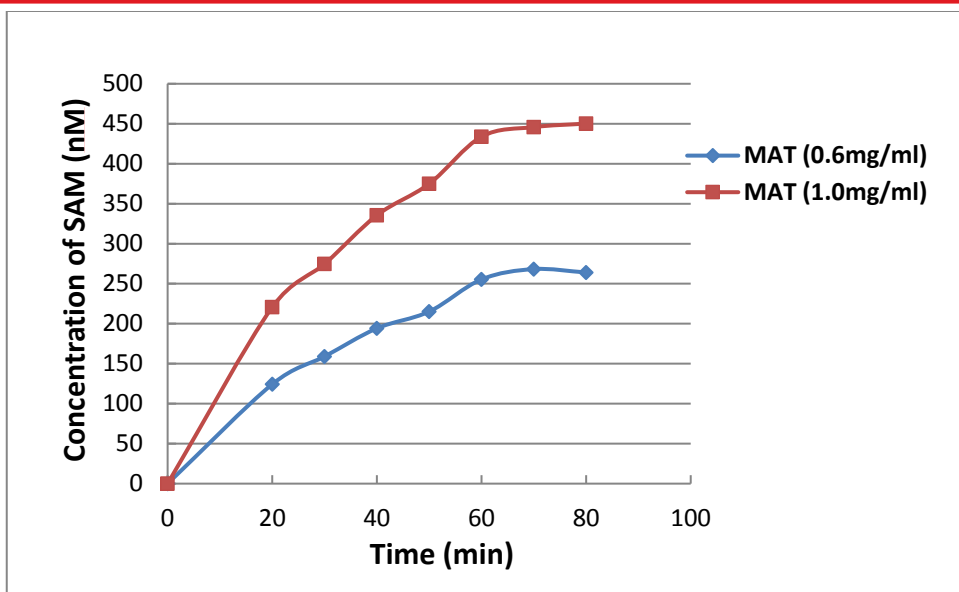
Document Release Date: July 24, 2017

Test	Specification	Result
Appearance	Clear liquid	Clear solution
Concentration (by UV Spectrophotometer)	0.5 - 1 mg/ml	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: 3nM; Plasma: 30-60nM	PBS: 3nM; Plasma: 30-60nM
Affinity (by cELISA, see details below)	$K_a \geq 10^9$ L/mol	$K_a = 7.68 \times 10^9$ L/mol $= 1.30 \times 10^{10}$ M

Specificity

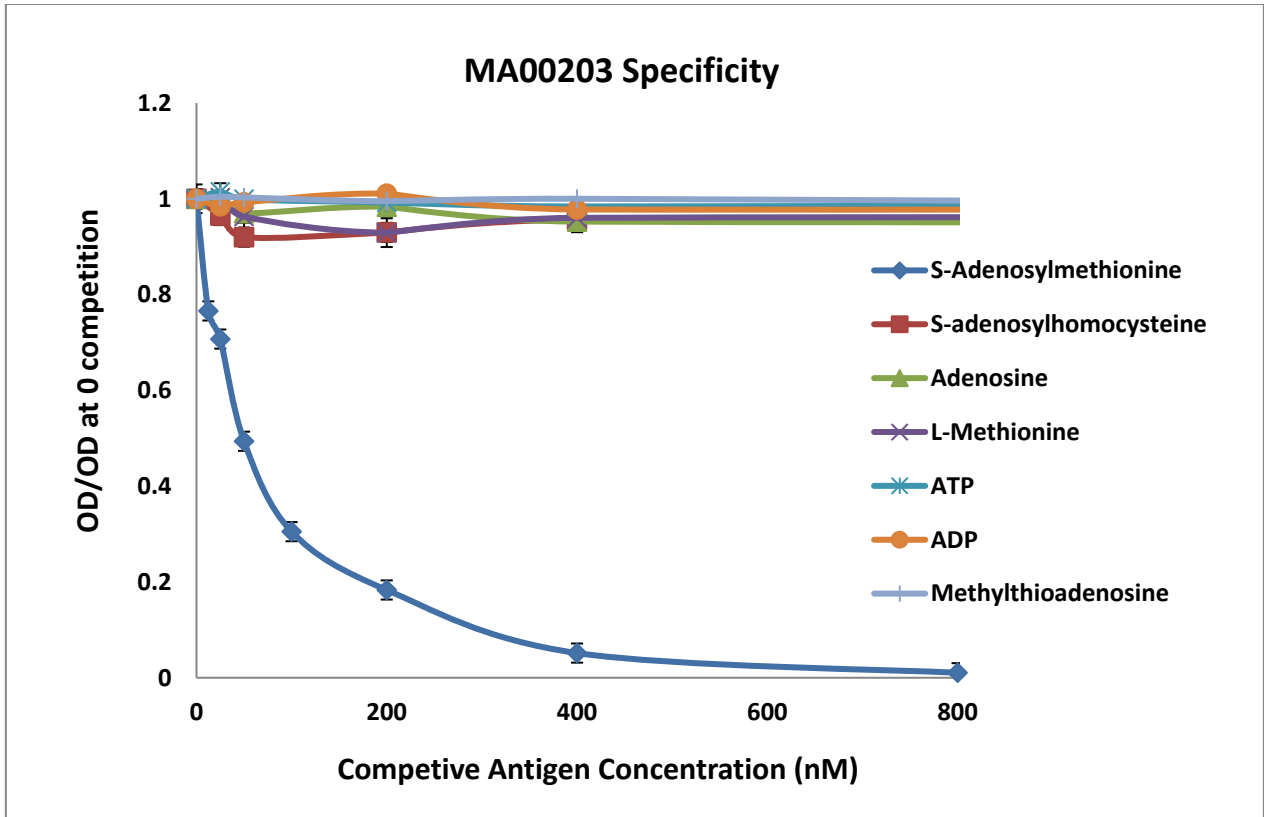
(1) Dosage-dependent competition was detected as a sample was added to a cELISA (Any SAM from a sample competes with the coated SAM heptan to bind HRP-conjugated antibody 118-18). The sample is the product of the following biochemical reaction: Methionine Adenosyltransferase (MAT) was added to methionine and adenosine triphosphate under an appropriate buffer at 37°C. It indicates that antibody 118-18 specifically binds physiologically produced SAM.

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(2) Cross reaction Figure below showed a competitive ELISA using anti-S-Adenosymethionine monoclonal antibody [118-18] (MA00203). The 0.1 $\mu\text{g/ml}$ of SAM coating standard (Cat # ACT00203) was coated into 96 wells. Serial dilution of SAM standard (Cat # AST00203), S-Adenosylhomocysteine (SAH), Adenosine (Ade), L-Methionine (Met), Methythioadenosine (MTA), Adenosine diphosphate (ADP), Adenosine triphosphate (ATP) and 1:15000 of MA00203 were added. HRP conjugated Goat anti-Mouse IgG antibody was used to develop the color.

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Sensitivity

Similar to clone 118-6

Affinity

Table 2 Anti-SAM Antibodies (118-18) Binding to Different Amount of Antigen

Dilution from 1mg/ml	Monoclonal anti-SAM 118-18 PLL-aza-SAM	
	0.05µg/ml	0.1µg/ml
1000	1.5046	2.5979
2000	1.4906	2.4437
4000	1.2725	2.3165
8000	1.1282	2.0831
16000	0.9172	1.7791

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32000	0.7316	1.442
64000	0.5371	1.149
128000	0.4012	0.9416

From Table 2, when coating antigen was at 0.05µg/ml, half the maximum OD was seen at about 1:32000. The corresponding antibody concentration was $[Ab]_t = (1\text{mg/ml}/160,000\text{g/mol})/32000 = 1.302 \times 10^{-10}\text{M}$, where 160,000 is the molecular weight of antibody. When coating antigen was at 0.1µg/ml, half the maximum OD was observed at about 1:48000. The corresponding antibody concentration was $[Ab] = (1\text{mg/ml}/160,000\text{g/mol})/48000 = 1.953 \times 10^{-10}\text{M}$.

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

$$K_a = (n-1)/2 * (n[Ab] - [Ab]_t) = 7.68 \times 10^9 \text{L/mol} = 1.30 \times 10^{-10}\text{M}$$

Jimmy Li
Quality Control Team

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