

## CERTIFICATE OF ANALYSIS

Product: Monoclonal anti-S-Adenosylmethionine (SAM) antibody clone 118-6

Catalog Number: MA00201

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

**Document Release Date:** July 24, 2017

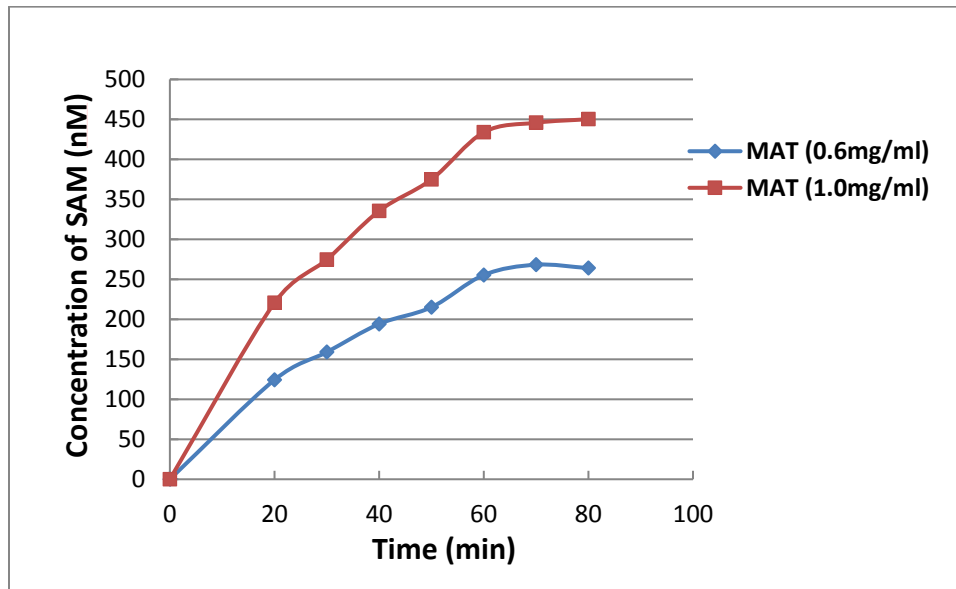
Test	Specification	Result
Appearance	Clear liquid	Clear solution
Concentration (by UV Spectrophotometer)	1 mg/ml	1.08mg/ml
Storage buffer	10mM PBS pH 7.4 (150 mM NaCl) , 10mg/ml BSA,0.02%NaN3, 50%Glycerol	10mM PBS pH 7.4 (150 mM NaCl) , 10mg/ml BSA,0.02%NaN3, 50%Glycerol
Stability at 37°C	Few hours	Same 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: 30nM
Affinity (by cELISA, see details below)	$K_a \geq 10^9$ L/mol	$K_a = 5.75 \times 10^9$ L/mol = $1.74 \times 10^{10}$ 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 hapten to bind HRP-conjugated antibody 118-6). 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

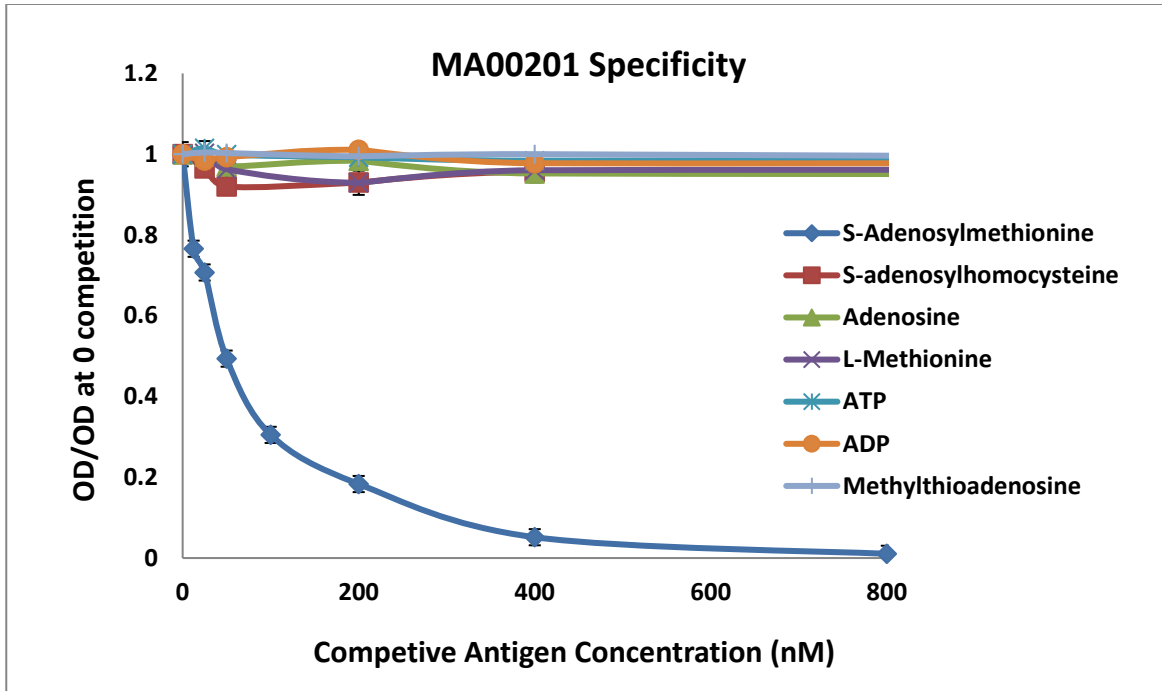
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118-6 specifically binds physiologically produced SAM.



(2) Cross reaction Figure below showed a competitive ELISA using anti-S-Adenosymethionine monoclonal antibody [118-6] (MA00201). 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 MA00201 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 curve measured from 0.1 μg/ml coated plate. ELISA was performed in triplicates. Mouse anti-SAM antibody 118-6 was used at 1:32000. 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).

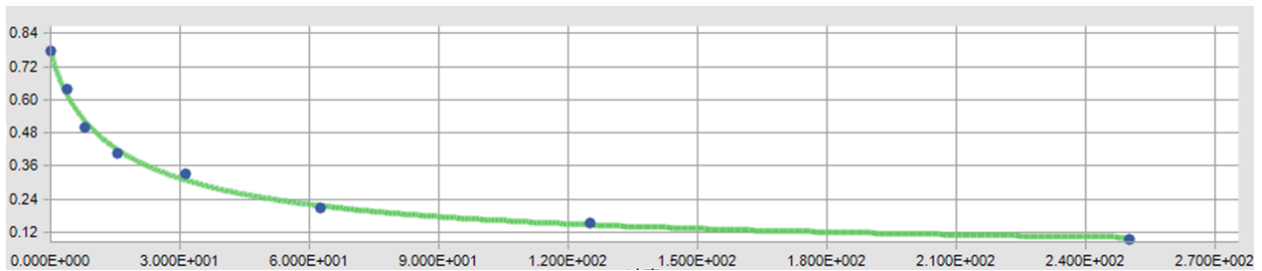


Table 1 Sensitivity by mouse anti-SAM antibody 118-6

SAM standard (nM)	OD450	OD450	OD450	Mean	SD
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Blank	0.0556	0.0808	0.0616	0.0660	0.0131
0	1.2189	1.2831	1.3030	1.2683	0.0439
6.25	1.110	1.0648	0.9757	1.0502	0.0683
12.5	0.885	0.8004	0.8737	0.8530	0.0459
25	0.6252	0.6435	0.6108	0.6265	0.0164
50	0.3838	0.3995	0.3782	0.3872	0.0110
Sensitivity	2.6 nM				

## Affinity

Table 2 Anti-SAM Antibodies (118-6) Binding to Different Amount of Antigen

Dilution from	Monoclonal anti-SAM 118-6 PLL-aza-SAM	
	0.1µg/ml	0.2µg/ml
1mg/ml		
100	4.4974	4.59425
200	4.2144	4.71145
400	4.0508	4.82100
800	3.9753	4.89140
1600	3.8187	4.86745
3200	3.6592	4.78590
6400	3.3300	4.77320
12800	3.2063	4.24435
25600	2.6556	3.60425
51200	1.8867	2.44935
102400	1.2460	1.60970
204800	0.7552	0.98320
409600	0.4396	0.54150
819200	0.2514	0.28610

From Table 2, when coating antigen was at 0.2µg/ml, half the maximum OD was seen at about 1:54800. The corresponding antibody concentration was  $[Ab] = (1\text{mg/ml}/160,000\text{g/mol})/54800 = 1.14 \times 10^{-10}\text{M}$ , 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 1:44300. The corresponding antibody concentration was  $[Ab]_t = (1\text{mg/ml}/160,000\text{g/mol})/44300 = 1.41 \times 10^{-10}\text{M}$ .

$$n = (0.2\mu\text{g/ml})/(0.1\mu\text{g/ml}) = 2$$

$$K_a = (n-1)/2 * (n[Ab] - [Ab]_t) = 5.75 \times 10^9 \text{L/mol} = 1.74 \times 10^{-10}\text{M}$$

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Jimmy Li  
Quality Control Team

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