

CERTIFICATE OF ANALYSIS

Product: Monoclonal anti-S-Adenosylhomocysteine (SAH) antibody clone 301-1

Catalog Number: MA00301

Lot Number: Lot#MP421804, Lot#MP421805, Lot# MP421608, Lot# MP422421, Lot#MP450216,
 Lot#MP450416, Lot#MP451607, Lot#MP451219, Lot#MP480213

Document Release Date: July 24, 2017

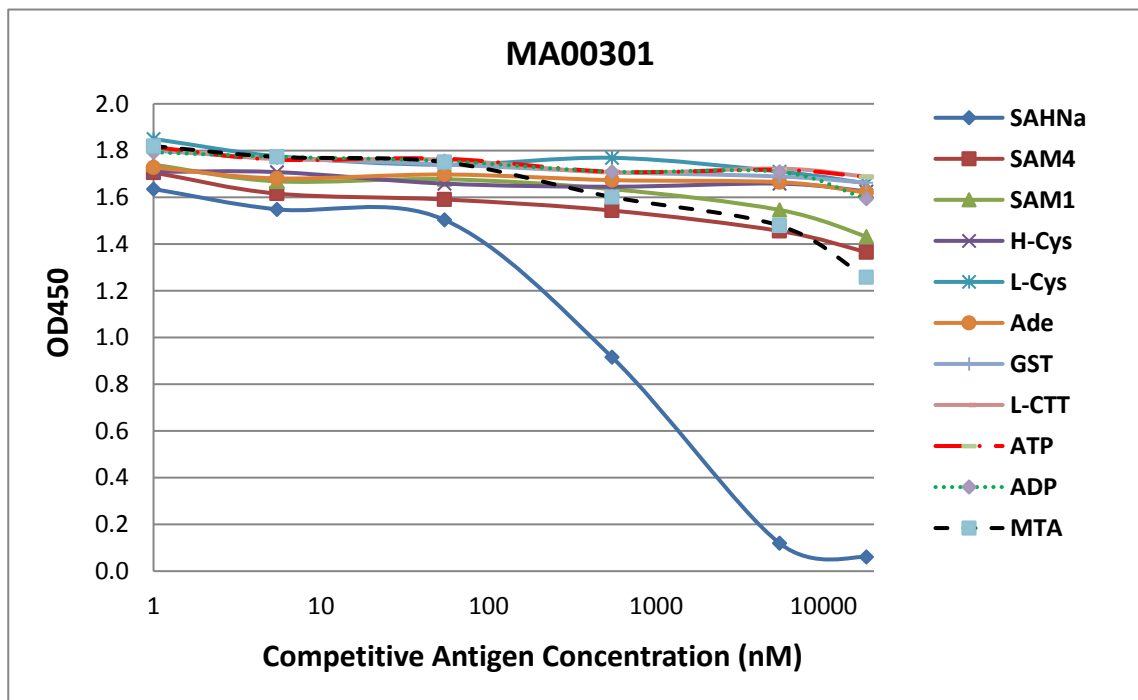
Test	Specification	Result
Appearance	Clear liquid	Clear solution
Concentration (by UV Spectrophotometer)	About 0.5 - 1 mg/ml	About 0.5 - 1mg/ml
Storage buffer	10mM PBS pH 7.4 (150 mM NaCl) , 10mg/ml BSA,0.02%NaN ₃ , 50%Glycerol	10mM PBS pH 7.4 (150 mM NaCl) , 10mg/ml BSA,0.02%NaN ₃ , 50%Glycerol
Stability 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 SAM <3%, other analogs < 1%	Cross reaction with SAM ~ 1.5%, other analogs < 1%
Sensitivity (by cELISA)	PBS: ~15nM Plasma: 62.5nM	PBS: ~15nM Plasma: 31.25 - 62.5nM
Affinity (by cELISA, see details below)	K _a >=10 ⁸ L/mol	K _a = 2.787 x 10 ⁸ L/mol = 3.6 x 10 ⁻⁹ M

Specificity

Figure below showed a competitive ELISA using anti-S-Adenosylhomocysteine monoclonal antibody [301-1] (MA00301). The 0.5 µg/ml of SAH-BSA was coated into 96 wells. Serial dilution of SAH standard

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(SAHNa), S-Adenosylmethionine (SAM4: Sigma-Aldrich Cat# A2408, SAM1: Aza-SAM Arthus Biosystems Cat# AST00201), Homocysteine (H-Cys), L-Cysteine (L-Cys), Adenosine (Ade), Glutathione (GST), L-Cystathionine (L-CTT), Methythioadenosine (MTA), ADP (adenosine diphosphate), ATP (adenosine triphosphate) and properly diluted MA00301 were added. HRP conjugated Goat anti-Mouse IgG antibody was used to develop the color. OD450 value was measured on each well. The relatively higher cross-reaction with SAM and MTA (<5%) should not be concerned as physiological levels of SAM and MTA are much lower than 1uM, at which level no cross reaction with the antibody is observed.



Sensitivity

Table 1 showed cELISA result with 0.5 µg/ml BSA-SAH coated micro-titer plate, HRP-anti-301-1 at dilution of 1:1000 in PBS incubation system. Different amounts of coating antigen, antibody, and incubating buffer will give slightly different minimum detection limits. The data showed the minimum detection limit, which was calculated by $OD(\text{when SAH Na} = 0) - 2 \times \text{Standard deviation} = 0.8697 - 2 \times 0.033747 = 0.8022$, was around 15.625nM.

Table 1 Standard curve in cELISA in PBS

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Standards(nM)	OD450	OD450	OD450	Mean	Stdev	Inhibition (%)
250	0.4116	0.4426	0.442	0.4321	0.017727	50.32%
125	0.5421	0.6358	0.5372	0.5717	0.055566	34.26%
62.5	0.6055	0.7115	0.6444	0.6538	0.053622	24.82%
31.25	0.7285	0.8199	0.7545	0.7676	0.047094	11.74%
15.625	0.8045	0.8273	0.7975	0.8098	0.015582	6.88%
7.8125	0.7602	0.8113	0.7708	0.7808	0.026969	10.22%
3.90625	0.7722	0.9144	0.7786	0.8217	0.080315	55.52%
0	0.877	0.8992	0.8329	0.8697	0.033747	0%

Affinity

Table 2 Anti-SAH Antibodies (301-1) Binding to Different Amount of Antigen

Dilution from 2mg/ml	BSA-SAH 1µg/ml	BSA-SAH 0.5µg/ml
25	4.5476	4.1062
50	4.775	3.5835
100	4.5725	3.2796
200	4.2861	2.9537
400	4.0144	2.6701
800	3.2889	2.2375
1600	2.4796	1.7737
3200	1.6566	1.2864
6400	0.9619	0.783
12800	0.4866	0.4297
25600	0.2505	0.2283
51200	0.1156	0.1137
102400	0.0652	0.0589
204800	0.0292	0.0296

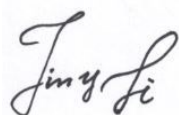
From Table 2, when coating antigen was at 1µg/ml, half the maximum OD was seen at about 1:1900. The corresponding antibody concentration was $[Ab] = (2\text{mg/ml}/160,000\text{g/mol})/1900 = 6.58 \times 10^{-9} \text{ M}$,

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where 160,000 is the molecular weight of antibody. When coating antigen was at 0.5µg/ml, half the maximum OD was observed at about 1:1100. The corresponding antibody concentration was $[Ab]_t = (2\text{mg/ml}/160,000\text{g/mol})/1100 = 1.136 \times 10^{-8} \text{ M}$.

$$n = (1\mu\text{g/ml})/(0.5\mu\text{g/ml}) = 2$$

$$K_a = (n-1)/2*(n[Ab] - [Ab]_t) = 2.787 \times 10^8 \text{ L/mol} = 3.6 \times 10^{-9} \text{ M}$$



Jimmy Li
Quality Control Team

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