

CERTIFICATE OF ANALYSIS

Product: Monoclonal anti-S-Adenosylhomocysteine (SAH) antibody clone 301-10

Catalog Number: MA00302

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

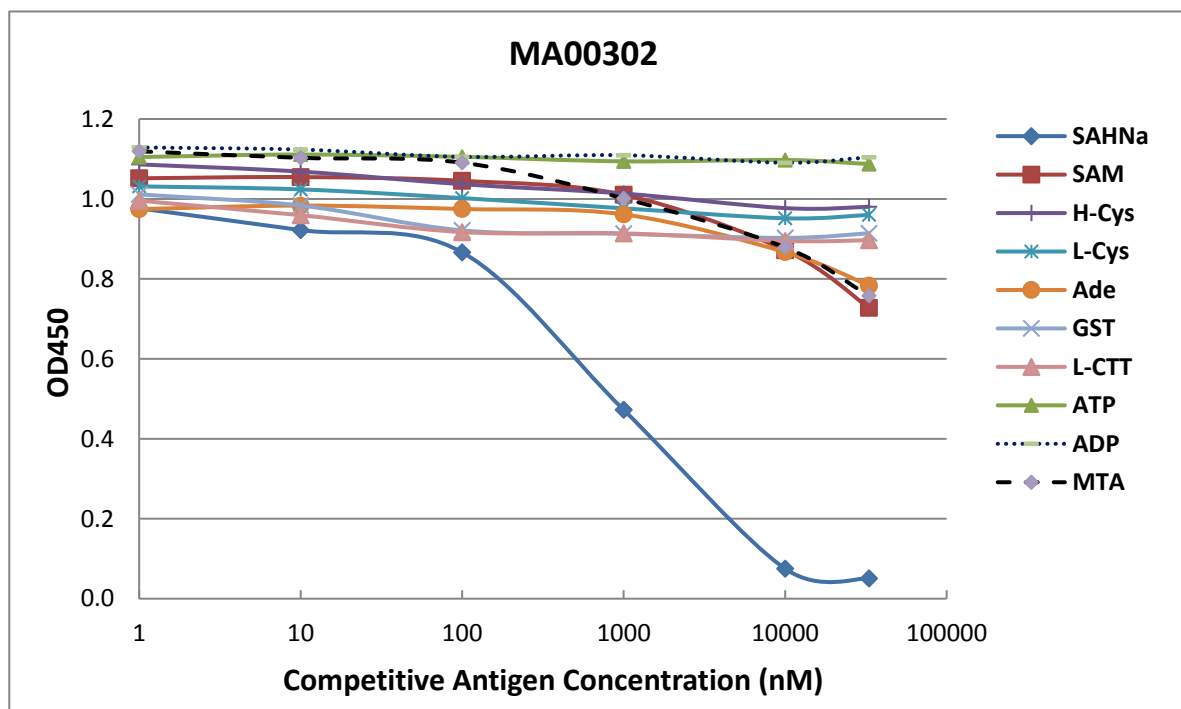
Document Release Date: July 24, 2017

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 = 4.2 x 10 ⁸ L/mol = 1.93 x 10 ⁻⁹ M

Specificity

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Figure below showed a competitive ELISA using anti-S-Adenosylhomocysteine monoclonal antibody [301-10]. The 0.5 µg/ml of SAH-BSA was coated into 96 wells. Serial dilution of SAH standard (SAHNa), S-Adenosylmethionine (SAM from Sigma-Aldrich Cat# A2408), 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 MA00302 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 1µM, at which level no cross reaction with the antibody is observed.



Sensitivity

Similar to 301-1

Affinity

Table 2 Anti-SAH Antibodies (301-10) Binding to Different Amount of Antigen

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Dilution from 1mg/ml	BSA-SAH at 0.5ug/ml	BSA-SAH at 0.25ug/ml
50	4.7308	3.4758
100	4.6129	3.2963
200	4.4614	3.1518
400	4.1938	2.9484
800	4.0873	2.7551
1600	3.557	2.4614
3200	2.783	2.0249
6400	2.1506	1.6019

From Table 2, when coating antigen was at 0.5µg/ml, half the maximum OD was seen at about 1:5600. The corresponding antibody concentration was $[Ab] = (1\text{mg/ml}/160,000\text{g/mol})/5600 = 1.116 \times 10^{-9} \text{ M}$, where 160,000 is the molecular weight of antibody. When coating antigen was at 0.25µg/ml, half the maximum OD was observed at about 1:6000. The corresponding antibody concentration was $[Ab]_t = (1\text{mg/ml}/160,000\text{g/mol})/6000 = 1.042 \times 10^{-9} \text{ M}$.

$$n = (0.5\mu\text{g/ml})/(0.25\mu\text{g/ml}) = 2$$

$$K_a = (n-1)/2 * (n[Ab] - [Ab]_t) = 4.2 \times 10^8 \text{ L/mol} = 1.93 \times 10^{-9} \text{ M}$$

Jimmy Li
Quality Control Team

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