

CERTIFICATE OF ANALYSIS

Product: Monoclonal anti-S-Adenosylhomocysteine (SAH) antibody clone 844-1

Catalog Number: MA00305

Lot Number: Lot# MP421608, Lot#MP480213, Lot#MP451607, Lot#MP451219

Document Release Date: July 24, 2017

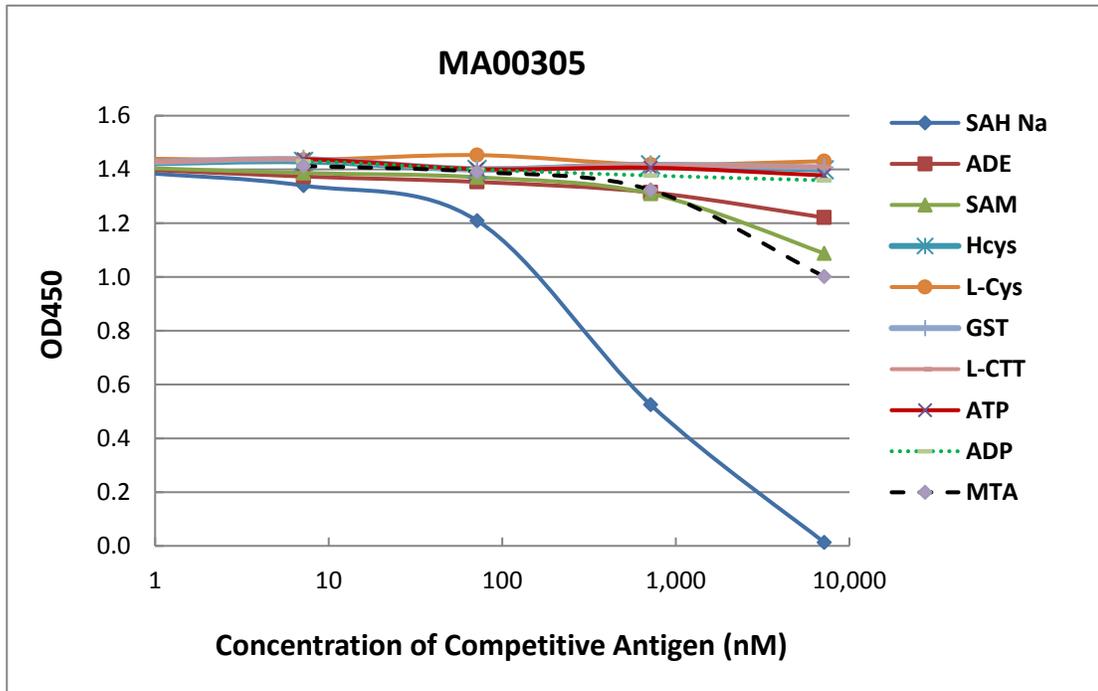
Test	Specification	Result
Appearance	Clear liquid	Clear solution
Concentration (by UV Spectrophotometer)	About 1.28 mg/ml	About 1.28 mg/ml
Storage buffer	10mM PBS pH 7.4 (150 mM NaCl), 0.02%NaN3	10mM PBS pH 7.4 (150 mM NaCl), 0.02%NaN3
Stability at 4°C	30 days	Same dilution in 48 days
Stability at -20°C	>1 year	>1 year
Stability at freeze and thaw	Avoid repeated freeze-and-thaw	No change in antigen-binding capability after freezer and thaw 10 times
Specificity	Cross reaction with SAM about <3%, other analogs < 1%	Cross reaction with SAM ~ 2.6%, other analogs < 1%
Sensitivity (by cELISA)	PBS: ~30nM Plasma: 62.5nM	PBS: ~30nM Plasma: 31.25 - 62.5nM
Affinity (by cELISA, see details below)	$K_a \geq 10^8$ L/mol	$K_a = 7.20 \times 10^9$ L/mol $= 1.39 \times 10^{-10}$ M
Titer (see curve below)	Adjust antibody at 1mg/ml	Suggested: 1:32,000 in cELISA

Specificity

Figure below showed a competitive ELISA using anti-S-Adenosylhomocysteine monoclonal antibody [844-1] (MA00305). The 0.2 µg/ml of SAH-BSA was coated into 96 wells. Serial dilution of SAH standard (SAHNa), S-Adosylmethionine (SAM: 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 MA00305 were added. HRP conjugated Goat anti-Mouse IgG antibody was used to develop the color. OD450 value was

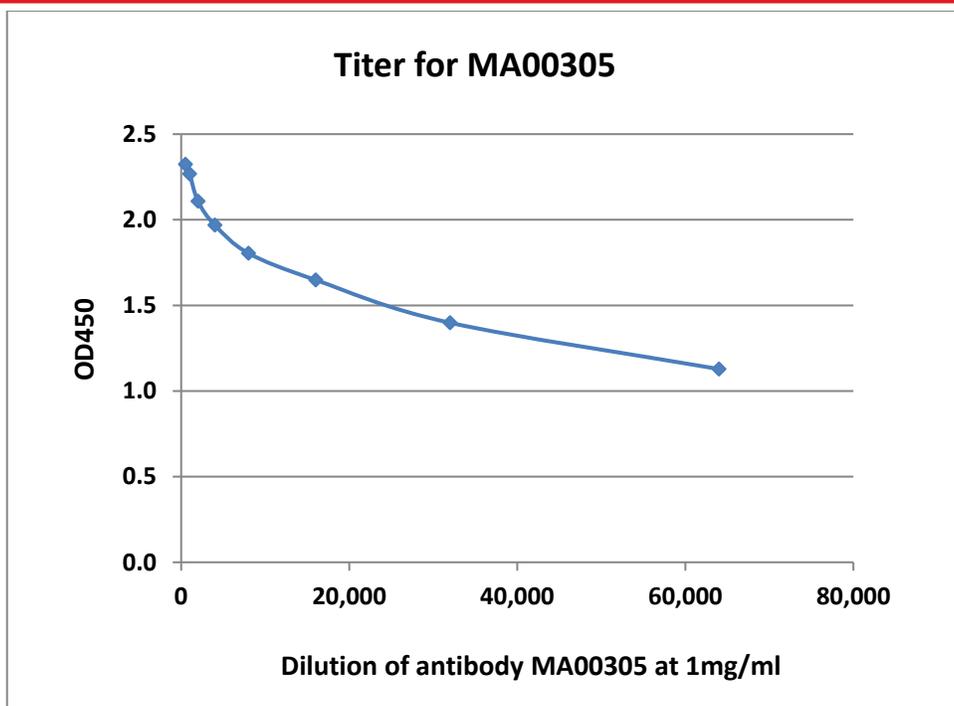
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measured on each well. The relatively higher cross-reaction with SAM and MTA (<5%) should not be concerned as physiological levels of SAM and MTA are much lower than 1uM, at which level no cross reaction with the antibody is observed.



Titer

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Affinity

Table 1 Anti-SAH Antibodies (844-1 at 1mg/ml) Binding to Different Amount of Antigen

Dilution	BSA-SAH 0.2ug/ml	BSA-SAH 0.4ug/ml
1000	2.94505	3.79275
2000	2.71675	3.6411
4000	2.51445	3.5291
8000	2.34215	3.33185
16000	2.0829	3.07145
32000	1.74925	2.5685
64000	1.28195	1.8321
128000	0.9474	1.3221

From Table 1, when coating antigen was at 0.4µg/ml, half the maximum OD was seen at about 1:64620. The corresponding antibody concentration was $[Ab] = (1\text{mg/ml}/160,000\text{g/mol})/64620 = 9.67 \times 10^{-11} \text{ M}$,

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where 160,000 is the molecular weight of antibody. When coating antigen was at 0.2µg/ml, half the maximum OD was observed at about 1:51000. The corresponding antibody concentration was $[Ab]_t = (1\text{mg/ml}/160,000\text{g/mol})/51000 = 1.225 \times 10^{-10} \text{ M}$.

$$n = 2$$

$$K_a = (n-1)/2*(n[Ab] - [Ab]_t) = 7.053 \times 10^9 \text{ L/mol}$$



Jimmy Li
Quality Control Team

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