

CERTIFICATE OF ANALYSIS

Product: Monoclonal anti-S-Adenosylhomocysteine (SAH) antibody clone 844-2

Catalog Number: MA00306

Lot Number: Lot#MP451219

Document Release Date: June 29, 2015

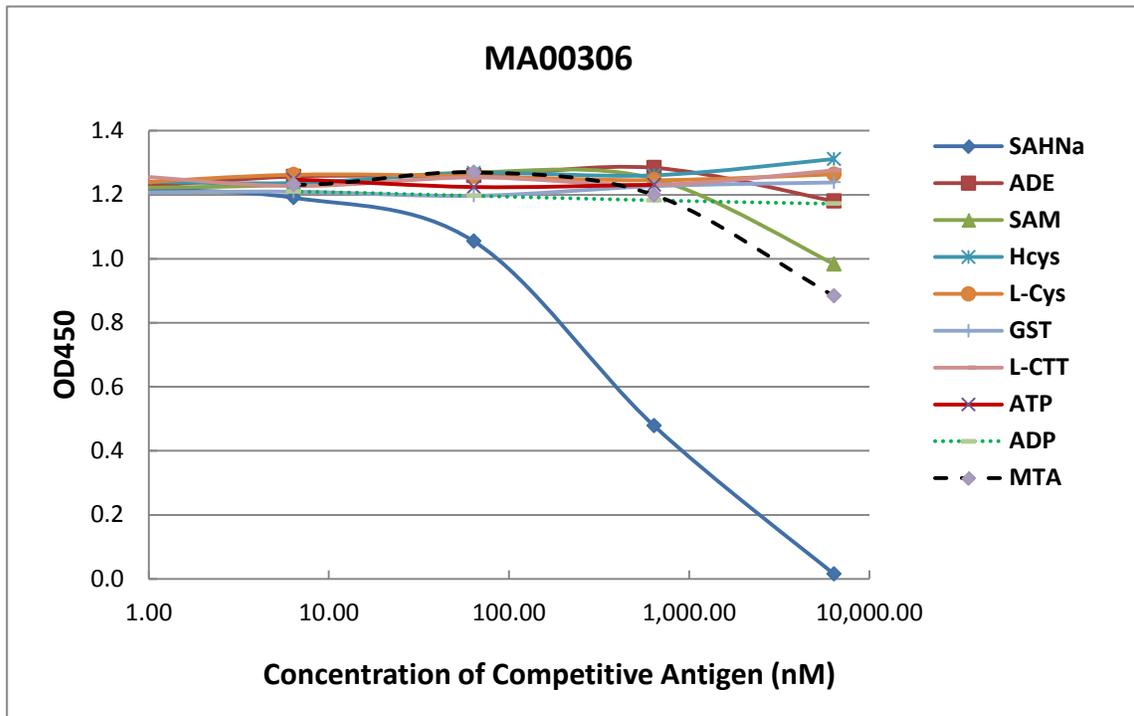
Test	Specification	Result
Appearance	Clear liquid	Clear solution
Concentration (by UV Spectrophotometer)	About 0.45mg/ml	About 0.45mg/ml
Storage buffer	10mM PBS pH 7.4 (150 mM NaCl), 0.02%NaN3	10mM PBS pH 7.4 (150 mM NaCl), 0.02%NaN3
Stability at 4°C	30 days	Same dilution in 48 days
Stability at -20°C	>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 ~ 2.1%, 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	$K_a = 2.34 \times 10^9$ L/mol $= 4.27 \times 10^{-10}$ M
Titer (see curve below)	Adjust antibody at 1mg/ml	Suggested: 1:10,000 in cELISA

Specificity

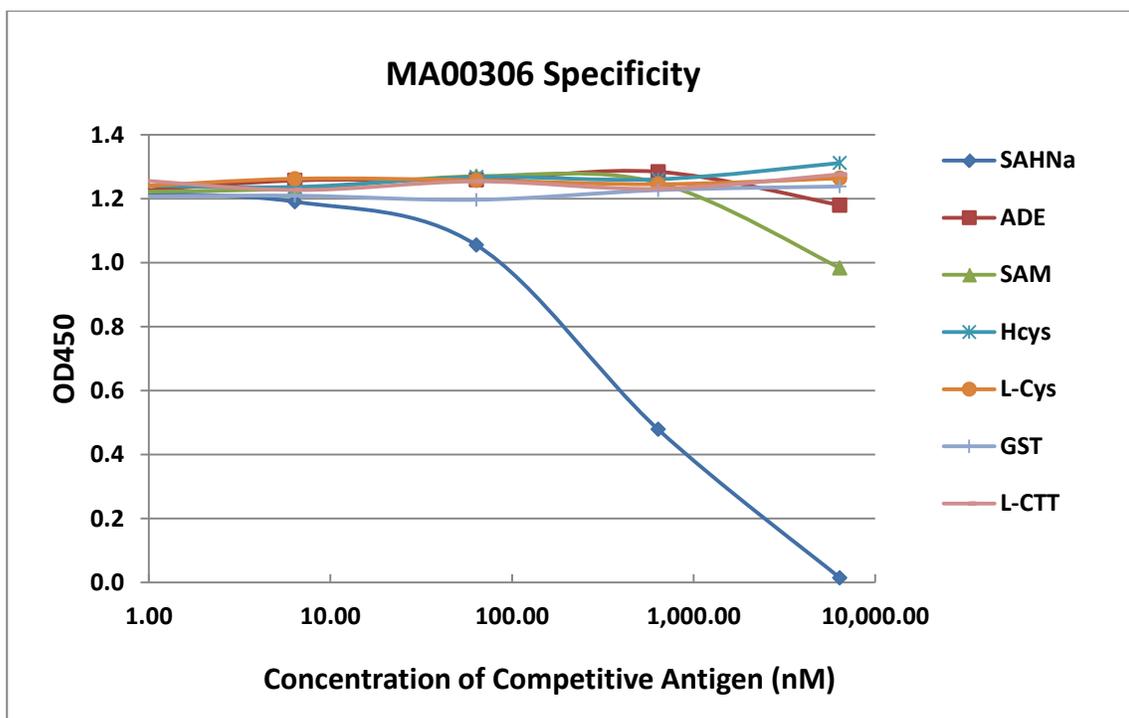
Figure below showed a competitive ELISA using anti-S-Adenosylhomocysteine monoclonal antibody [844-2] (MA00306). The 0.2 µg/ml of SAH-BSA was coated into 96 wells. Serial dilution of SAH standard (SAHNa), S-Adosylmethionine (SAM: Sigma-Aldrich Cat# A2408), Homocysteine (H-Cys), L-Cysteine (L-

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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 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.

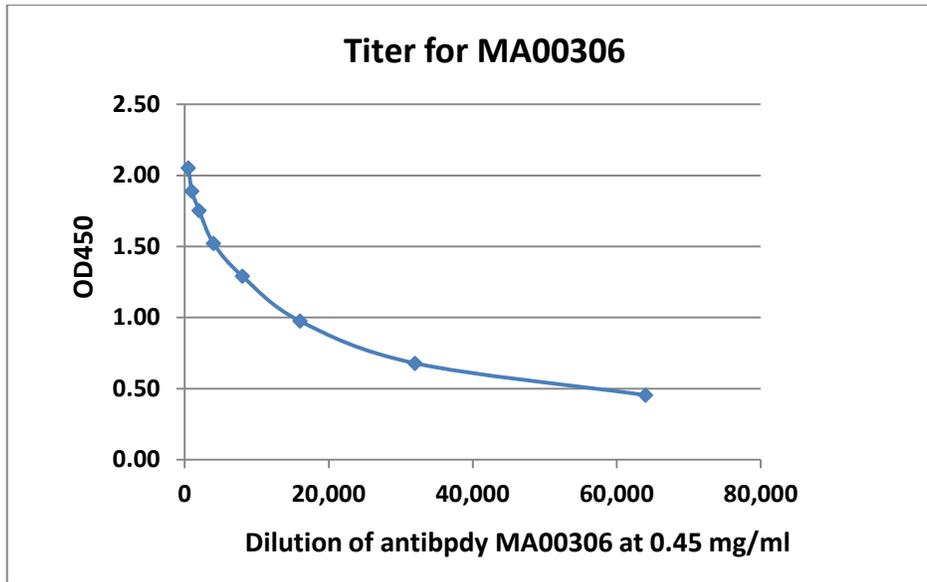


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Titer

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Affinity

Table 1 Anti-SAH Antibodies (844-2 at 0.45mg/ml) Binding to Different Amount of Antigen

Dilution	BSA-SAH 0.1ug/ml	BSA-SAH 0.2ug/ml
250	1.7700	2.55865
500	1.6057	2.358
1000	1.4772	2.19125
2000	1.3468	2.0004
4000	1.1313	1.70235
8000	0.8507	1.2667
16000	0.6090	0.84835
32000	0.4302	0.5697

From Table 1, when coating antigen was at 0.2µg/ml, half the maximum OD was seen at about 1:9569. The corresponding antibody concentration was $[Ab] = (0.45\text{mg/ml}/160,000\text{g/mol})/9569 = 2.939 \times 10^{-10}$

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M, where 160,000 is the molecular weight of antibody. When coating antigen was at 0.1 μ g/ml, half the maximum OD was observed at about 1:7510. The corresponding antibody concentration was $[Ab]_t = (0.45\text{mg/ml}/160,000\text{g/mol})/7510 = 3.745 \times 10^{-10}$ M.

n = 2

$K_a = (n-1)/2 * (n[Ab] - [Ab]_t) = 2.34 \times 10^9$ L/mol



Jimmy Li

Quality Control Team

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