

CERTIFICATE OF ANALYSIS

Product: Monoclonal anti-S-Adenosylhomocysteine (SAH) antibody clone 839-6

Catalog Number: MA00307

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

Document Release Date: July 24, 2017

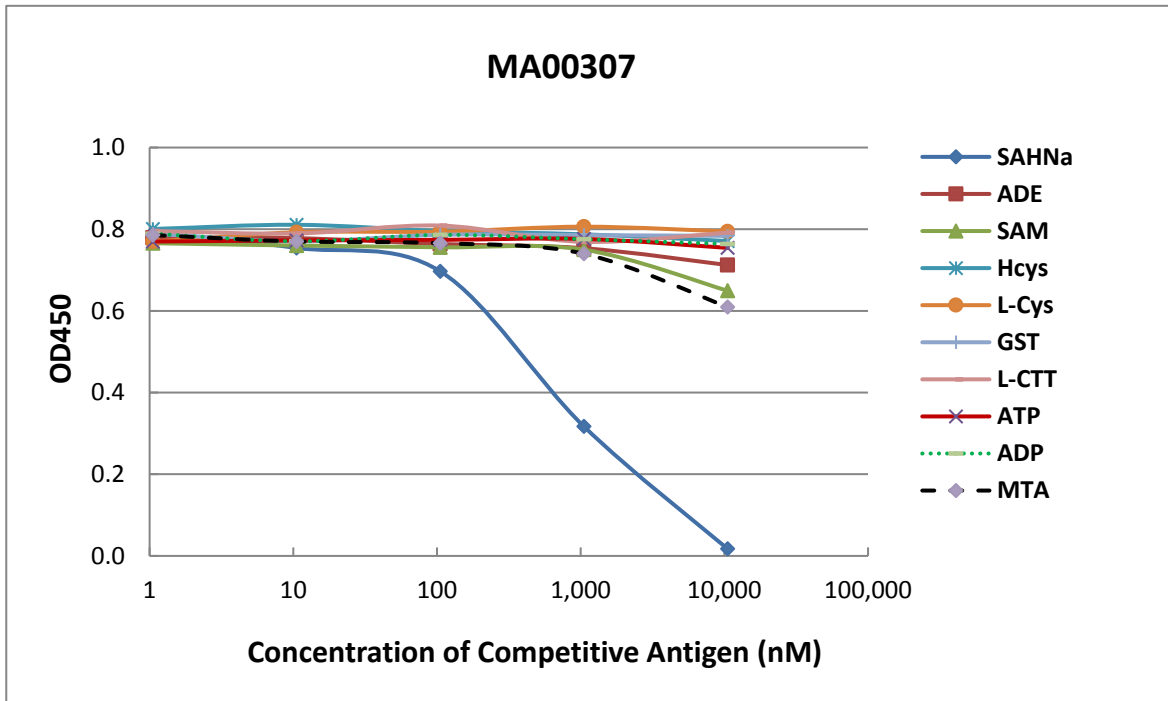
| Test | Specification | Result |
|---|---|---|
| Appearance | Clear liquid | Clear solution |
| Concentration (by UV Spectrophotometer) | About 1.3mg/ml | About 1.3mg/ml |
| Storage buffer | 10mM PBS pH 7.4 (150 mM NaCl), 0.02%NaN3 | 10mM PBS pH 7.4 (150 mM NaCl), 0.02%NaN3 |
| Stability at 4°C | 30 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 ~ 1.3%, 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 | $K_a = 7.38 \times 10^9$ L/mol $= 1.35 \times 10^{-10}$ M |
| Titer (see curve below) | Adjust antibody at 1mg/ml | Suggested: 32,000 in cELISA |

Specificity

Figure below showed a competitive ELISA using anti-S-Adenosylhomocysteine monoclonal antibody [839-6] (MA00307). 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

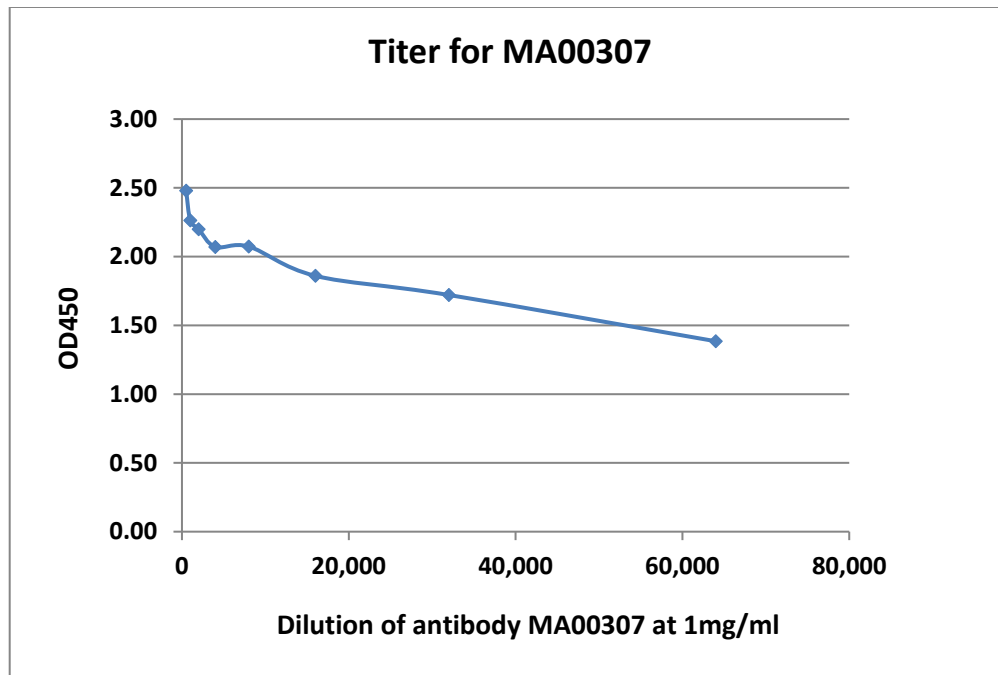
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measured on each well. The relatively higher cross-reaction with SAM and MTA (<5%) should not be concerned as physiological levels of SAM and MTA are much lower than 1uM, at which level no cross reaction with the antibody is observed.



Titer

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Affinity

Table 1 Anti-SAH Antibodies (839-6 at 1mg/ml) Binding to Different Amount of Antigen

| Dilution | BSA-SAH 0.1ug/ml | BSA-SAH 0.2ug/ml |
|----------|------------------|------------------|
| 4000 | 1.9792 | 2.8577 |
| 8000 | 1.8630 | 2.7525 |
| 16000 | 1.7730 | 2.54815 |
| 32000 | 1.5913 | 2.2704 |
| 64000 | 1.3102 | 1.7912 |
| 128000 | 0.9864 | 1.2551 |
| 256000 | 0.6384 | 0.82805 |
| 512000 | 0.4205 | 0.5177 |

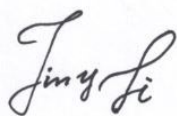
From Table 1, when coating antigen was at 0.2µg/ml, half the maximum OD was seen at about 1:110400. The corresponding antibody concentration was $[Ab] = (1\text{mg/ml}/160,000\text{g/mol})/ 110400 = 5.66 \times 10^{-11} \text{ M}$, where 160,000 is the molecular weight of antibody. When coating antigen was at 0.1µg/ml,

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half the maximum OD was observed at about 1: 137500. The corresponding antibody concentration was $[Ab]_t = (1\text{mg/ml}/160,000\text{g/mol})/ 137500 = 4.545 \times 10^{-11} \text{ M}$.

$n = 2$

$K_a = (n-1)/2 * (n[Ab] - [Ab]_t) = 7.38 \times 10^9 \text{ L/mol}$



Jimmy Li
Quality Control Team

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