KAMIYA BIOMEDICAL COMPANY

Pig Serum Amyloid A (SAA) ELISA

For the quantitative determination of serum amyloid A (SAA) in pig serum or plasma

Cat. No. KT-748

For Research Use Only.

PRODUCT INFORMATION

Pig Serum Amyloid A (SAA) ELISA
Cat. No. KT-748

PRODUCT
The **K-ASSAY®** Pig Serum Amyloid A (SAA) ELISA is an enzyme immunoassay for the quantitative determination of serum amyloid A (SAA) in pig serum or plasma. For research use only.

INTRODUCTION
SAA, a protein of ~12 kDa, is a positive acute phase reactant that circulates in blood mostly associated with high density lipoprotein (HDL). In pigs, basal serum levels are ~5 µg/mL. Levels can reach 500 µg/mL during infection.

PRINCIPLE
The assay uses two different peptide-specific pig SAA antibodies; one for solid phase immobilization and the other, conjugated to horseradish peroxidase (HRP), for detection. Serum or plasma samples are first denatured by heating for 1 hour at 60℃. The denaturing step dissociates SAA from interfering factors. Subsequently, the denatured samples are diluted. Calibrators and diluted samples are incubated, in the microtiter wells, together with HRP conjugate for one hour. This results in SAA molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate. TMB is added and incubated for 20 minutes. If SAA is present a blue color develops. Color development is stopped by addition of Stop solution, changing the color to yellow, and absorbance is measured at 450 nm. The concentration of SAA is proportional to absorbance and is derived from a calibration curve.

COMPONENTS
- SAA antibody coated 96-well plate (12 x 8 well strips)
- HRP conjugate stock
- SAA calibrator stock (lyophilized)
- Wash Buffer (20X), 50 mL
- Diluent, 50 mL
- TMB Reagent, 11 mL
- Stop Solution, 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED
- Pipettors and tips
- Distilled or de-ionized water
- Polypropylene or glass tubes
- Vortex mixer
- Water bath
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm
- Curve fitting software

GENERAL INSTRUCTIONS
1. All reagents should be allowed to reach room temperature before use.
2. Reliable and reproducible results will be obtained when the assay is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. Laboratory temperature will influence absorbance readings. Our ELISA kits are calibrated using shaking incubators set at 150 rpm and 25℃. Performance of the assay at lower temperatures will result in lower absorbance values.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20X stock. Prior to use, dilute the contents of the bottle (50 mL) with 950 mL of distilled or de-ionized water.

SAMPLE PREPARATION

Denaturation

1. Dispense 100 µL of each serum or plasma sample into a polypropylene microcentrifuge tube and tightly seal.
2. Incubate the samples at 60°C in a water bath for one hour.

Dilution

Dilute the denatured samples at least 400-fold with the diluent provided with the kit. Optimum dilutions must be empirically determined.

CALIBRATOR PREPARATION

1. Reconstitute the SAA stock by adding 0.20 mL of H_2O. Mix gently several times before use. The stock does not require heat treatment.
2. Label 7 polypropylene tubes as 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 ng/mL.
3. Into the tube labeled 50 ng/mL, pipette 469.9 µL of diluent. Then add 30.1 µL of SAA calibrator stock and mix gently. This provides the 50 ng/mL calibrator.
4. Dispense 250 µL of diluent into the tubes labeled 25, 12.5, 6.25, 3.13, 1.56 and 0.78 ng/mL.
5. Pipette 250 µL of the 50 ng/mL SAA calibrator into the tube labeled 25 ng/mL and mix. This provides the 25 ng/mL SAA calibrator.
6. Similarly prepare the remaining calibrators by two-fold serial dilution.

Unused stock should be stored frozen at or below -20°C if future use is intended.

HRP CONJUGATE PREPARATION

The anti-pig SAA HRP conjugate is provided as a concentrated stock. Shortly before use, dilute the stock by mixing 1.0 µL of HRP conjugate stock with 1.0 mL of diluent for each 8-well strip.

DILUENT

The diluent is specially formulated for measurement of SAA in pig serum or plasma. It is provided ready to use. Do not substitute other buffers.

ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the holder. Unused strips should be stored in the re-sealed bag with desiccant at 4°C for future use.
2. Dispense 100 µL of calibrators and samples into the wells (we recommend that calibrators and samples be run in duplicate).
3. Add 100 µL of HRP-conjugate into each well.
4. Incubate on a plate shaker at 150 rpm and 25°C for one hour.
5. Empty and wash the microtiter wells 5x with 1X wash solution using a plate washer (400 µL/well).
6. Strike the wells sharply onto adsorbent paper or paper towels to remove all residual droplets.
7. Dispense 100 µL of TMB into each well.
8. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
9. After 20 minutes, stop the reaction by adding 100 µL of Stop solution to each well.
10. Gently mix. It is important to make sure that all the blue color changes to yellow.
11. Read absorbance at 450 nm with a plate reader within 5 minutes.

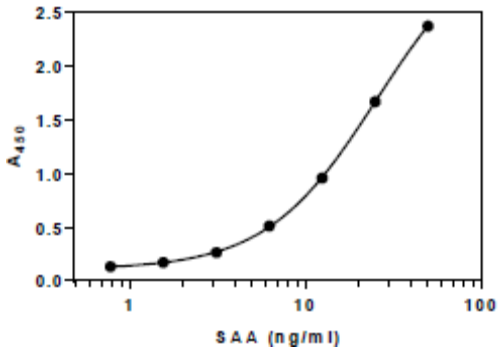
CALCULATION OF RESULTS

1. Using curve fitting software, construct a calibration curve by plotting absorbance values of the calibrators versus $\log_{10}$ of the concentration.
2. Fit the calibration curve to a four-parameter logistic regression (4PL) equation (x axis = $\log_{10}$ concentration) and determine the concentration of the samples from the calibration curve (remember to derive the antilog).
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the serum or plasma sample.
4. If the A_{450} values of samples fall outside the calibration curve, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration curve with absorbance at 450 nm on the Y-axis against SAA concentrations on the X-axis is shown below. This curve is for illustration only.

SAA (ng/mL)	A_{450}
50	2.374
25	1.670
12.5	0.959
6.25	0.514
3.13	0.266
1.56	0.174
0.78	0.134



STORAGE

The SAA calibrator stock and the HRP conjugate stock should be stored at or below -20℃ for optimum stability. The remainder of the kit should be stored at 4℃ and the microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable until the expiration date.

FOR RESEARCH USE ONLY

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PRODUCT INFORMATION

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PRODUCT
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INTRODUCTION
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PRINCIPLE
The assay uses two different peptide-specific pig SAA antibodies; one for solid phase immobilization and the other, conjugated to horseradish peroxidase (HRP), for detection. Serum or plasma samples are first denatured by heating for 1 hour at 60℃. The denaturing step dissociates SAA from interfering factors. Subsequently, the denatured samples are diluted. Calibrators and diluted samples are incubated, in the microtiter wells, together with HRP conjugate for one hour. This results in SAA molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate. TMB is added and incubated for 20 minutes. If SAA is present a blue color develops. Color development is stopped by addition of Stop solution, changing the color to yellow, and absorbance is measured at 450 nm. The concentration of SAA is proportional to absorbance and is derived from a calibration curve.

COMPONENTS
- SAA antibody coated 96-well plate (12 x 8 well strips)
- HRP conjugate stock
- SAA calibrator stock (lyophilized)
- Wash Buffer (20X), 50 mL
- Diluent, 50 mL
- TMB Reagent, 11 mL
- Stop Solution, 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED
- Pipettors and tips
- Distilled or de-ionized water
- Polypropylene or glass tubes
- Vortex mixer
- Water bath
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm
- Curve fitting software

GENERAL INSTRUCTIONS
1. All reagents should be allowed to reach room temperature before use.
2. Reliable and reproducible results will be obtained when the assay is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. Laboratory temperature will influence absorbance readings. Our ELISA kits are calibrated using shaking incubators set at 150 rpm and 25℃. Performance of the assay at lower temperatures will result in lower absorbance values.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20X stock. Prior to use, dilute the contents of the bottle (50 mL) with 950 mL of distilled or de-ionized water.

SAMPLE PREPARATION

Denaturation

1. Dispense 100 µL of each serum or plasma sample into a polypropylene microcentrifuge tube and tightly seal.
2. Incubate the samples at 60°C in a water bath for one hour.

Dilution

Dilute the denatured samples at least 400-fold with the diluent provided with the kit. Optimum dilutions must be empirically determined.

CALIBRATOR PREPARATION

1. Reconstitute the SAA stock by adding 0.20 mL of H_2O. Mix gently several times before use. The stock does not require heat treatment.
2. Label 7 polypropylene tubes as 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 ng/mL.
3. Into the tube labeled 50 ng/mL, pipette 469.9 µL of diluent. Then add 30.1 µL of SAA calibrator stock and mix gently. This provides the 50 ng/mL calibrator.
4. Dispense 250 µL of diluent into the tubes labeled 25, 12.5, 6.25, 3.13, 1.56 and 0.78 ng/mL.
5. Pipette 250 µL of the 50 ng/mL SAA calibrator into the tube labeled 25 ng/mL and mix. This provides the 25 ng/mL SAA calibrator.
6. Similarly prepare the remaining calibrators by two-fold serial dilution.

Unused stock should be stored frozen at or below -20°C if future use is intended.

HRP CONJUGATE PREPARATION

The anti-pig SAA HRP conjugate is provided as a concentrated stock. Shortly before use, dilute the stock by mixing 1.0 µL of HRP conjugate stock with 1.0 mL of diluent for each 8-well strip.

DILUENT

The diluent is specially formulated for measurement of SAA in pig serum or plasma. It is provided ready to use. Do not substitute other buffers.

ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the holder. Unused strips should be stored in the re-sealed bag with desiccant at 4°C for future use.
2. Dispense 100 µL of calibrators and samples into the wells (we recommend that calibrators and samples be run in duplicate).
3. Add 100 µL of HRP-conjugate into each well.
4. Incubate on a plate shaker at 150 rpm and 25°C for one hour.
5. Empty and wash the microtiter wells 5x with 1X wash solution using a plate washer (400 µL/well).
6. Strike the wells sharply onto adsorbent paper or paper towels to remove all residual droplets.
7. Dispense 100 µL of TMB into each well.
8. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
9. After 20 minutes, stop the reaction by adding 100 µL of Stop solution to each well.
10. Gently mix. It is important to make sure that all the blue color changes to yellow.
11. Read absorbance at 450 nm with a plate reader within 5 minutes.

CALCULATION OF RESULTS

1. Using curve fitting software, construct a calibration curve by plotting absorbance values of the calibrators versus $\log_{10}$ of the concentration.
2. Fit the calibration curve to a four-parameter logistic regression (4PL) equation (x axis = $\log_{10}$ concentration) and determine the concentration of the samples from the calibration curve (remember to derive the antilog).
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the serum or plasma sample.
4. If the A_{450} values of samples fall outside the calibration curve, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration curve with absorbance at 450 nm on the Y-axis against SAA concentrations on the X-axis is shown below. This curve is for illustration only.

SAA (ng/mL)	A_{450}
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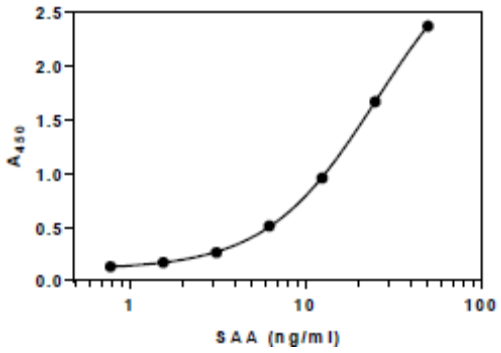

STORAGE

The SAA calibrator stock and the HRP conjugate stock should be stored at or below -20℃ for optimum stability. The remainder of the kit should be stored at 4℃ and the microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable until the expiration date.

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PRINCIPLE
The assay uses two different peptide-specific pig SAA antibodies; one for solid phase immobilization and the other, conjugated to horseradish peroxidase (HRP), for detection. Serum or plasma samples are first denatured by heating for 1 hour at 60℃. The denaturing step dissociates SAA from interfering factors. Subsequently, the denatured samples are diluted. Calibrators and diluted samples are incubated, in the microtiter wells, together with HRP conjugate for one hour. This results in SAA molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate. TMB is added and incubated for 20 minutes. If SAA is present a blue color develops. Color development is stopped by addition of Stop solution, changing the color to yellow, and absorbance is measured at 450 nm. The concentration of SAA is proportional to absorbance and is derived from a calibration curve.

COMPONENTS
- SAA antibody coated 96-well plate (12 x 8 well strips)
- HRP conjugate stock
- SAA calibrator stock (lyophilized)
- Wash Buffer (20X), 50 mL
- Diluent, 50 mL
- TMB Reagent, 11 mL
- Stop Solution, 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED
- Pipettors and tips
- Distilled or de-ionized water
- Polypropylene or glass tubes
- Vortex mixer
- Water bath
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm
- Curve fitting software

GENERAL INSTRUCTIONS
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2. Reliable and reproducible results will be obtained when the assay is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. Laboratory temperature will influence absorbance readings. Our ELISA kits are calibrated using shaking incubators set at 150 rpm and 25℃. Performance of the assay at lower temperatures will result in lower absorbance values.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20X stock. Prior to use, dilute the contents of the bottle (50 mL) with 950 mL of distilled or de-ionized water.

SAMPLE PREPARATION

Denaturation

1. Dispense 100 µL of each serum or plasma sample into a polypropylene microcentrifuge tube and tightly seal.
2. Incubate the samples at 60°C in a water bath for one hour.

Dilution

Dilute the denatured samples at least 400-fold with the diluent provided with the kit. Optimum dilutions must be empirically determined.

CALIBRATOR PREPARATION

1. Reconstitute the SAA stock by adding 0.20 mL of H_2O. Mix gently several times before use. The stock does not require heat treatment.
2. Label 7 polypropylene tubes as 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 ng/mL.
3. Into the tube labeled 50 ng/mL, pipette 469.9 µL of diluent. Then add 30.1 µL of SAA calibrator stock and mix gently. This provides the 50 ng/mL calibrator.
4. Dispense 250 µL of diluent into the tubes labeled 25, 12.5, 6.25, 3.13, 1.56 and 0.78 ng/mL.
5. Pipette 250 µL of the 50 ng/mL SAA calibrator into the tube labeled 25 ng/mL and mix. This provides the 25 ng/mL SAA calibrator.
6. Similarly prepare the remaining calibrators by two-fold serial dilution.

Unused stock should be stored frozen at or below -20°C if future use is intended.

HRP CONJUGATE PREPARATION

The anti-pig SAA HRP conjugate is provided as a concentrated stock. Shortly before use, dilute the stock by mixing 1.0 µL of HRP conjugate stock with 1.0 mL of diluent for each 8-well strip.

DILUENT

The diluent is specially formulated for measurement of SAA in pig serum or plasma. It is provided ready to use. Do not substitute other buffers.

ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the holder. Unused strips should be stored in the re-sealed bag with desiccant at 4°C for future use.
2. Dispense 100 µL of calibrators and samples into the wells (we recommend that calibrators and samples be run in duplicate).
3. Add 100 µL of HRP-conjugate into each well.
4. Incubate on a plate shaker at 150 rpm and 25°C for one hour.
5. Empty and wash the microtiter wells 5x with 1X wash solution using a plate washer (400 µL/well).
6. Strike the wells sharply onto adsorbent paper or paper towels to remove all residual droplets.
7. Dispense 100 µL of TMB into each well.
8. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
9. After 20 minutes, stop the reaction by adding 100 µL of Stop solution to each well.
10. Gently mix. It is important to make sure that all the blue color changes to yellow.
11. Read absorbance at 450 nm with a plate reader within 5 minutes.

CALCULATION OF RESULTS

1. Using curve fitting software, construct a calibration curve by plotting absorbance values of the calibrators versus $\log_{10}$ of the concentration.
2. Fit the calibration curve to a four-parameter logistic regression (4PL) equation (x axis = $\log_{10}$ concentration) and determine the concentration of the samples from the calibration curve (remember to derive the antilog).
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the serum or plasma sample.
4. If the A_{450} values of samples fall outside the calibration curve, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration curve with absorbance at 450 nm on the Y-axis against SAA concentrations on the X-axis is shown below. This curve is for illustration only.

SAA (ng/mL)	A_{450}
50	2.374
25	1.670
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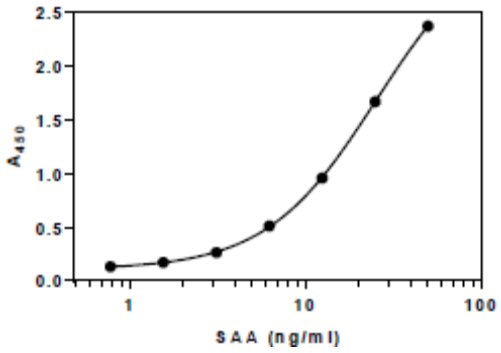

STORAGE

The SAA calibrator stock and the HRP conjugate stock should be stored at or below -20℃ for optimum stability. The remainder of the kit should be stored at 4℃ and the microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable until the expiration date.

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COMPONENTS
- SAA antibody coated 96-well plate (12 x 8 well strips)
- HRP conjugate stock
- SAA calibrator stock (lyophilized)
- Wash Buffer (20X), 50 mL
- Diluent, 50 mL
- TMB Reagent, 11 mL
- Stop Solution, 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED
- Pipettors and tips
- Distilled or de-ionized water
- Polypropylene or glass tubes
- Vortex mixer
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WASH SOLUTION PREPARATION

The wash solution is provided as a 20X stock. Prior to use, dilute the contents of the bottle (50 mL) with 950 mL of distilled or de-ionized water.

SAMPLE PREPARATION

Denaturation

1. Dispense 100 µL of each serum or plasma sample into a polypropylene microcentrifuge tube and tightly seal.
2. Incubate the samples at 60°C in a water bath for one hour.

Dilution

Dilute the denatured samples at least 400-fold with the diluent provided with the kit. Optimum dilutions must be empirically determined.

CALIBRATOR PREPARATION

1. Reconstitute the SAA stock by adding 0.20 mL of H_2O. Mix gently several times before use. The stock does not require heat treatment.
2. Label 7 polypropylene tubes as 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 ng/mL.
3. Into the tube labeled 50 ng/mL, pipette 469.9 µL of diluent. Then add 30.1 µL of SAA calibrator stock and mix gently. This provides the 50 ng/mL calibrator.
4. Dispense 250 µL of diluent into the tubes labeled 25, 12.5, 6.25, 3.13, 1.56 and 0.78 ng/mL.
5. Pipette 250 µL of the 50 ng/mL SAA calibrator into the tube labeled 25 ng/mL and mix. This provides the 25 ng/mL SAA calibrator.
6. Similarly prepare the remaining calibrators by two-fold serial dilution.

Unused stock should be stored frozen at or below -20°C if future use is intended.

HRP CONJUGATE PREPARATION

The anti-pig SAA HRP conjugate is provided as a concentrated stock. Shortly before use, dilute the stock by mixing 1.0 µL of HRP conjugate stock with 1.0 mL of diluent for each 8-well strip.

DILUENT

The diluent is specially formulated for measurement of SAA in pig serum or plasma. It is provided ready to use. Do not substitute other buffers.

ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the holder. Unused strips should be stored in the re-sealed bag with desiccant at 4°C for future use.
2. Dispense 100 µL of calibrators and samples into the wells (we recommend that calibrators and samples be run in duplicate).
3. Add 100 µL of HRP-conjugate into each well.
4. Incubate on a plate shaker at 150 rpm and 25°C for one hour.
5. Empty and wash the microtiter wells 5x with 1X wash solution using a plate washer (400 µL/well).
6. Strike the wells sharply onto adsorbent paper or paper towels to remove all residual droplets.
7. Dispense 100 µL of TMB into each well.
8. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
9. After 20 minutes, stop the reaction by adding 100 µL of Stop solution to each well.
10. Gently mix. It is important to make sure that all the blue color changes to yellow.
11. Read absorbance at 450 nm with a plate reader within 5 minutes.

CALCULATION OF RESULTS

1. Using curve fitting software, construct a calibration curve by plotting absorbance values of the calibrators versus $\log_{10}$ of the concentration.
2. Fit the calibration curve to a four-parameter logistic regression (4PL) equation (x axis = $\log_{10}$ concentration) and determine the concentration of the samples from the calibration curve (remember to derive the antilog).
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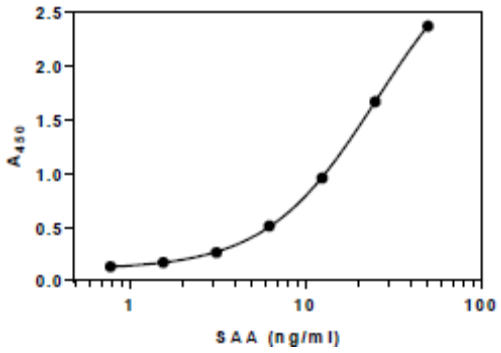

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