Pig IgA ELISA

INTRODUCTION

The pig IgA ELISA kit is designed for measurement of IgA (immunoglobulin A) in pig serum or plasma. The assay uses anti-pig IgA for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-pig IgA for detection. Both capture and detection antibodies were cross-absorbed on pig IgM and IgG agarose, thereby ensuring specificity for IgA. Cross-reactivity with immunoglobulins from other species has not been investigated.

IgA is present in pig serum at concentrations ranging from 0.36 to 2.25 mg/ml. Levels in colostrum are ~25 mg/ml 1 hour after parturition but decline to ~ 5 mg/ml within 24 hours (ref 1).

PRINCIPLE OF THE ASSAY

Samples are diluted and incubated in the microtiter wells for 45 minutes alongside prepared pig IgA standards. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. IgA molecules are thus sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies and TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of IgA is proportional to the optical density of the test sample and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti pig IgA coated 96-well plate (12 strips of 8 wells)
- HRP Conjugate Reagent, 11 ml
- Reference Standard (lyophilized)
- 20x Wash Solution, 50 ml
- 10x Immunoglobulin Diluent (25 ml)
- TMB Reagent (One-Step) 11 ml
- Stop Solution (1N HCl), 11 ml

Materials required but not provided:

- Precision pipettes and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker mixing speed of ~150 rpm
- Plate washer
- Plate reader with an optical density range of 0-4 at 450nm
- Graph paper (PC graphing software is optional)

STORAGE

The test kit will remain stable for six months from the date of purchase provided that the components are stored as described above. The microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air.

GENERAL INSTRUCTIONS

1. Please read and understand the instructions thoroughly before using the kit.
2. All reagents should be allowed to reach room temperature (25^{o}C) before use.
3. Optimum results are achieved if, at each step, reagents are pipetted into the wells of the microtiter plate within 5 minutes.

DILUENT PREPARATION

The diluent is provided as a 10x stock. Prior to use estimate the final volume of diluent required for your assay and dilute one (1) volume of the 10x stock with nine (9) volumes of distilled or deionized water.

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 deionized water.

STANDARD PREPARATION

1. The pig IgA standard is provided as a lyophilized stock. Reconstitute as described on the vial label ***(the reconstituted standard is stable at 4^{o}C for at least 24 hours but should be aliquoted and frozen at -20^{o}C after reconstitution if future use is intended)***.
2. Label 8 polypropylene or glass tubes as 300, 150, 75, 37.5, 18.75, 9.38, 4.69 and 0 ng/ml.
3. Into the tube labeled 300 ng/ml, pipette the volume of diluent detailed on the IgA standard vial label. Then add the indicated volume of IgA stock (shown on the IgA standard vial label) and mix gently. This provides the 300 ng/ml standard.
4. Dispense 250 µl of diluent into the tubes labeled 150, 75, 37.5, 18.75, 9.38, 4.69 and 0 ng/ml.
5. Prepare a 150 ng/ml standard by diluting and mixing 250 µl of the 300 ng/ml standard with 250 µl of diluent in the tube labeled 150 ng/ml.
6. Similarly prepare the 75, 37.5, 18.75, 9.38, 4.69 ng/ml standards by serial dilution.

SAMPLE PREPARATION

General Note: IgA is typically present in pig serum or plasma at concentrations of approximately 1 mg/ml. In order to obtain values within range of the standard curve, we suggest that samples be diluted 20,000 fold using the following procedure for each sample to be tested:

1. Dispense 198 µl and 497.5 µl of 1x diluent into separate tubes.
2. Pipette and mix 2 µl of the serum/plasma sample into the tube containing 198 µl of diluent. This provides a 100 fold diluted sample.
3. Mix 2.5 µl of the 100 fold diluted sample with the 497.5 µl of diluent in the second tube. This provides a 20,000 fold dilution of the sample.

4. Repeat this procedure for each sample to be tested

Optimal dilutions of colostrum, tissue extracts and other fluids should be determined empirically.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standards and diluted samples into the wells (we recommend that standards and samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (25°C)[1] for 45 minutes.
4. Aspirate the contents of the microtiter wells and wash the wells 5 times with 1x wash solution using a plate washer (400 µl/well). The entire wash procedure should be performed as quickly as possible.
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash buffer.
6. Add 100 µl of HRP conjugate reagent into each well.
7. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (25°C) for 45 minutes.
8. Wash as detailed in 4 to 5 above.
9. Dispense 100 µl of TMB Reagent into each well.
10. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (25°C) for 20 minutes.
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix. *It is important to make sure that all the blue color changes to yellow.*
13. Read the optical density at 450 nm with a microtiter plate reader *within 5 minutes*.

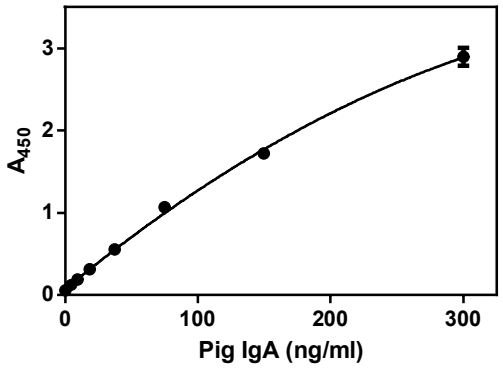
CALCULATION OF RESULTS

1. Calculate the average absorbance values (A_{450}) for each set of reference standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of IgA in ng/ml from the standard curve.
4. Multiply the derived concentrations by the dilution factor to determine the actual concentration of IgA in the sample.
5. Ideally, PC graphing software should be used for the above steps. We find good fits of standard data to a second order polynomial equation.
6. If the OD_{450} values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with optical density readings at 450nm on the Y axis against IgA concentrations on the X axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

IgA (ng/ml)	A_{450}
300	2.897
150	1.719
75	1.067
37.5	0.552
18.75	0.314
9.38	0.184
4.69	0.122
0	0.057



LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of and in accordance with the instructions detailed above.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

REFERENCES

1. Markowska-Daniel I, Pomorska-Mol M and Pejsak Z.. Dynamic changes of immunoglobulin concentrations in pig colostrum and serum around parturition.. Polish J. Vet. Sci. 13(1): 21-27 (2010).

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For technical assistance please email us at techsupport@lifediagnostics.com

[1] The ELISA was validated using a shaking incubator at 25°C and 150 rpm. Lower temperatures and/or mixing speeds will give lower absorbance values.