ELISA Kit Components	Amount	Part No.
Anti-Pig Albumin Microwell Strip Plate	8-well strips (12)	9001
Pig Albumin Positive Control	0.65 ml	9002
Pig Albumin Standard 5 ng/ml	0.65 ml	9003B
Pig Albumin Standard 10 ng/ml	0.65 ml	9003C
Pig Albumin Standard 20 ng/ml	0.65 ml	9003D
Pig Albumin Standard 50 ng/ml	0.65 ml	9003E
Pig Albumin Standard 100 ng/ml	0.65 ml	9003F
Anti-Pig Albumin HRP Conjugate (100X)	0.15 ml	9004
Sample Diluent Concentrate (20X)	10 ml	SD-20T
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-9000

Other ELISA kits available from ADI

Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgG3, IgG2b, IgM, Leptin, Acrp30, CRP, Haptoglobin, TNF-alpha, VEGF, SAP.

Human: BD-1, BD-2, BD-3, NP-1 **and**: Adiponectin (Acrp30 and gAcrp30), Albumin, Aldosterone, AFP, Angiogenin, Angiopoietin-2, beta-2M, C-peptide, CRP, Cox-2, Ferritin, PSA, fPSA, GH, IgG, IgM, IgA, Insulin, NSE, CA125, CA199, CA242, PAP, SHBG, LH, FSH, TSH, T3, T4, and Steroid ELISA kits (cortisol, E2, testosterone, progesterone etc).

Rat: Albumin, CRP, IgG, IgM, Alpha 1 Acid glycoprotein

Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin

Monkey: IgM, IgG, IgA, CRP, IgE
Chicken: IgY(G), IgM, Ovalbumin
Rabbit: CRP, IgG
Pig: IgG, IgM

Dog: CRP, IgG, IgM
Cat: IgG, IgM
Goat: IgG

For more details please consult our web site (www.4adi.com) or contact us by email (service@4adi.com).

Instruction Manual No. M-9000

Pig Albumin

ELISA Kit Cat. #9000

For Quantitative Determination of Pig Albumin in Solution

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INTENDED USE

The Pig Albumin ELISA Kit is an in vitro immunoassay for research use in the quantification of Pig albumin circulating in serum or in other appropriately qualified samples from tissue fluids (e.g., saliva, mucosa) or in cultures of pig cells.

INTRODUCTION

Albumin, synthesized in the liver, is the protein of the highest concentration in plasma. Albumin transports many small molecules in the blood (for example, bilirubin, calcium, progesterone, and drugs) and is of prime importance in maintaining the osmotic pressure of the circulatory system.

Liver disease, kidney disease, and malnutrition are the major causes of low albumin. A diseased liver produces insufficient albumin. Diseased kidneys sometimes lose large amounts of albumin into the urine faster than the liver can produce it (this is termed nephritic syndrome).

Plasma albumin concentration is an important indicator of nutritional status. Low concentrations pre-surgery increase the risk of post-operative wound re-opening, seroma formation, and infection. Albumin levels are also dependant on the state of hydration of the body, whereby dehydration raises albumin levels, which return to normal when the dehydration is corrected. This sensitivity to hydration state results in wide fluctuations in circulating albumin levels.

PRINCIPLE OF THE TEST

The Pig Albumin ELISA kit is based on the binding of Pig albumin in samples to two antibodies: one immobilized on the microtiter wells and the other conjugated to horseradish peroxidase (HRP) enzyme. After a washing step, chromogenic substrate is added and color is developed by the enzymatic reaction of HRP on the TMB substrate which is directly proportional to the amount of albumin present in the sample. Stopping Solution is added to terminate the reaction and absorbance at 450nm is then measured using an ELISA microtiter well reader. The concentration of albumin in samples and control is calculated from a curve of standards containing known concentrations of albumin.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{o}C until the expiration date printed on the kit label. Stabilities of the working solutions are indicated under Reagent Preparation.

PERFORMANCE CHARACTERISTICS & EXPECTED RESULTS

Specificity

The antibodies used in this kit have been shown by immunoelectrophoresis and ELISA to react specifically with albumin and have essentially no reactivity with immunoglobulins or any other pig serum proteins.

Serum from the following species showed no significant reactivity at 1:10k dilution: human, monkey, hamster, guinea pig, bovine, horse, sheep, goat, dog, cat, rabbit, or chicken; also 10% neonatal bovine serum.

Normal Range

Assay of albumin in a limited number of stored sera from individual adult pigs ranged from 23 to 37 mg/ml (median = 30 mg/ml). Each laboratory should determine expected values of its own testing population.

QUALITY CONTROL

Standard Curve
The signal generated by the standards should be continuously increasing in OD from the lowest Standard to the highest Standard with a difference greater than 1.2 OD. Non-continuously increasing or low signals may indicate problems with technique, protocol directions, and/or reagent preparation, use, or stability. A Negative Diluent Control should be of lower signal than the lowest standard. Do not rely on results generated from an assay with these issues.

Sample Controls
Each lab should assay internal control samples which represent the lab's expected sample population and that are maintained stabilized. A Negative Diluent Control should also be run.

Technique
Accurate and reproducible assay results rely on good lab technique regarding pipetting, plate washing, and handling of samples and reagents.

Equipment
Precision of results relies on uniform and effective washing techniques; an automatic washer is recommended. ELISA reader and pipettes should be properly calibrated.

CALCULATIONS

The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, pig albumin concentrations may be determined as follows:

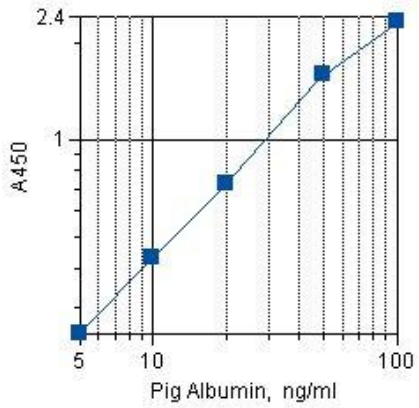
1. Calculate the mean OD of duplicate samples.
2. On graph paper, plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of Pig albumin (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
3. The Pig albumin concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
4. Multiply the values obtained for the samples by the dilution factor of each sample.
5. Samples producing signals higher than the 100 ng/ml standard should be further diluted and re-assayed.

TYPICAL RESULTS

The following data are for illustration purposes only. A complete standard curve should be run in every assay to determine sample values.

Wells	Standards, Control & Samples	A450 nm	Pig albumin ng/ml
1A, B	**Negative Diluent Control**	0.08	0
1C, D	5 ng/ml **Standard**	0.25	5
1E, F	10 ng/ml **Standard**	0.43	10
1G, H	20 ng/ml **Standard**	0.73	20
2A, B	50 ng/ml **Standard**	1.58	50
2C, D	100 ng/ml **Standard**	2.31	100
2E, F	**Positive Serum Control** [Value: 24 - 46 ng/ml]	1.12	33
2G, H	**Sample** [Diluted 1:500k]	1.31	40
	Calculated: 500k-fold dilution x 40 ng/ml = **20** mg/ml in serum		

A typical assay Standard Curve (do not use for calculating sample values)



KIT CONTENTS

Ready For Use: Store as indicated on labels.

Component	Part #	Amt	Contents
Anti-Pig Albumin Microwell Strip Plate	9001	8-well strips (12)	Coated with purified anti- Pig albumin antibodies. Return unused strips to the pouch with desiccant; re-seal and store refrigerated.
Positive Control [Albumin] range on label	9002	0.65 ml	Pig serum with stated albumin concentration range; diluted in buffer with protein, detergents, and antimicrobial as stabilizers.
Pig Albumin Standards			
5 ng/ml 10 ng/ml 20 ng/ml 50 ng/ml 100 ng/ml	9003B 9003C 9003D 9003E 9003F	0.65 ml 0.65 ml 0.65 ml 0.65 ml 0.65 ml	Five (5) vials, each containing pig serum calibrated using purified pig albumin; diluted in buffer with protein, detergents, and antimicrobial as stabilizers.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	1% sulfuric acid.

To Be Reconstituted: Store as indicated.

Component	Instructions for Use
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8ºC until the kit lot expires or is used up.
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume, 10ml, to 1L with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely.
Anti-Pig albumin-HRP Conjugate Concentrate (100x) Part No. 9004, 0.15ml	Peroxidase conjugated anti-Pig albumin antibody in buffer with protein, detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8ºC.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Antibody-HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate and Sample Diluent Concentrate; 200ml to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices and avoid ingestion or contact of any reagent with skin, eyes, or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid, and BND can be requested or obtained from the ADI website.

SPECIMEN COLLECTION AND HANDLING

Culture medium, serum, and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference.

For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature.

For **other samples**, including tissue culture media, clarify the sample by centrifugation and/or filtration prior to dilution in Working Sample Diluent. If samples will not be assayed immediately , stored refrigerated for up to a week, or frozen for long-term storage. Avoid freeze-thaw cycles.

ASSAY PROCEDURE

Bring all reagents to room temperature (18-30°C) equilibration (at least 30 minutes).

DILUTE Serum Samples in Working Sample Diluent. Dilutions of about 1:500k are appropriate for most normal Pig sera. For accuracy, three dilution steps are recommended, as follows:

1) 10ul serum + 990ul diluent = [1:100],
2) 10ul [1:200] + 990ul diluent = [1:10k],
3) 20ul [1:10k] + 980ul diluent = **[1:500k].**

DO NOT dilute the Standards or Positive Control Serum.

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. Set-up

- Determine the number of wells for the assay run. Duplicates are recommended, to include 10 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand about 1-5 minutes before sample addition.
- Aspirate or dump the liquid and pat the plate dry on a paper towel.

2. 1st Incubation [100ul - 60min; 4 washes]

- Add 100ul of standards, samples, and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

3. 2nd Incubation [100ul - 30min; 5 washes]

- Add 100ul of Working Anti-Pig albumin-HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

4. Substrate Incubation [100ul - 15min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

5. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

6. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.