Pig IgG ELISA Core Kit, pink-ONE

1. Catalog No. K0231094P

2. Quantity 1000 tests

3. Storage 4°C

4. Description Pig IgG ELISA Core Kit contains the core reagents required for performing quantitative measurement of Pig IgG levels from samples including serum, plasma or other biological fluids in a sandwich ELISA format.

5. Standard range 500 - 7.8 ng/ml

6. Kit Contents

Component	Description	Conc.	Amount	Working Dilution
Coating Antibody	Affinity purified Goat anti- Pig IgG	1 mg/ml	0.1 ml	1/1000
Detection Antibody	HRP conjugated Goat anti-Pig IgG	1 mg/ml	0.1 ml	1:3,000-1:10,000
Standard Protein	Rat Reference Serum	16.5 mg/ml	0.1 ml	500–7.8 ng/ml
Prestained Color Development Reagent	pink-ONE TMB solution	-	100 ml	Ready for use

7. Recommended Materials

- ELISA microplates
- Coating Buffer
- Tween-20
- Blocking Solution
- Stop Solution
- PBS
-

Note: Recommended for use with ELISA Starter Kit (K0331001 or K0331002).

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8. Reagent Preparations

*** Prepare the following buffers. All preparations should be mixed thoroughly and warmed up at room temperature prior to use.**

1. **Coating Buffer:** 50 mM Carbonate-Bicarbonate Buffer, pH 9.6
   Resolve the coating material (sample and standard) in the Coating Buffer to make 1ug/ml (1-10ug/ml).

2. **Assay Diluent:** PBS, 1% BSA, pH 7.4
   Dilute Sample/Standard/Antibody in Assay Diluent. Or use Washing Solution or Blocking Solution instead of Assay Diluent to help prevent non-specific binding.

3. **Washing Solution:** PBS, 0.05% Tween-20, pH 7.4
   Add 1ml Tween-20 (50%) to 1 Liter PBS and mix well.

4. **Blocking Solution:** PBS, 1% BSA, pH 7.4.

9. Cautions

1. Store all solutions at 4°C and keep them from contamination.

2. All samples and kit reagents should be at room temperature (20-25°C) prior to use.

3. Vigorous washing of the plate after incubation steps is essential to obtaining low background values.

4. Dissolve antigen, standard and antibody perfectly.

5. Use clean pipet tips for each transfer to avoid cross contamination.

6. Stop solution (H_2SO_4) is a caustic material. Eye, hand, face, and clothing protection should be worn when handling this material.

7. Sodium Azide should not be added to any of the buffers.

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10. ELISA Protocol

1. Coating

1) Dilute Coating Antibody at a ratio of 1:1000 with Coating Buffer and coat each well with 100 ul of diluted Coating Antibody.
2) Incubate for overnight at 4°C.
3) Aspirate the wells to remove liquid and wash the plate 3-5 times with Washing Solution.

Note: Complete removal of liquid at each step is essential to good performance. Do not dry the well completely and so immediately go on next step.

2. Blocking

1) Add 200 ul of Blocking Solution to each well.
2) Incubate at room temperature for at least 1 hour.
3) Aspirate the wells to remove liquid and wash the plate 3-5 times with Washing Solution.

3. Standards and Samples

1) Dilute the standards and samples in Assay Diluent at 1:2 serial dilutions as follows:

Step	Dilution Method	Standard conc.
Step A	2 ul of Standard + 2.26 ml of Assay Diluent	10,000 ng/ml
Step B	0.05 ml of Step A + 0.95 ml of Assay Diluent	500 ng/ml
Step C	0.5 ml of Step B + 0.5 ml of Assay Diluent	250 ng/ml
Step D	0.5 ml of Step C + 0.5 ml of Assay Diluent	125 ng/ml
Step E	0.5 ml of Step D + 0.5 ml of Assay Diluent	62.5 ng/ml
Step F	0.5 ml of Step E + 0.5 ml of Assay Diluent	31.25 ng/ml
Step G	0.5 ml of Step F + 0.5 ml of Assay Diluent	15.625 ng/ml
Step H	0.5 ml of Step G + 0.5 ml of Assay Diluent	7.8 ng/ml

Note: Dilute the samples, based on the expected concentration of the analyte, to fall within the concentration range of the standards.

2) Transfer 100 ul of standard or sample to assigned wells.
3) Incubate at room temperature for at least 1 hour.
4) Aspirate the wells to remove liquid and wash the plate 3-5 times with Washing Solution.

4. Detection Antibody

1) Dilute the Detection Antibody in Assay Diluent.
2) Recommended starting dilution is 1:5,000 with a range of 1:3,000 to 1:10,000.

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Note: Adjustments in dilution may be needed depending on substrate used, incubation time, and age of kit.

3) Add 100 ul per well and incubate at room temperature for at least 1 hour.
4) Aspirate the wells to remove liquid and wash the plate 3-5 times with Washing Solution.

5. Color Reaction and Reading

1) Add 100 ul of pink-ONE TMB Color Development Reagent to each well. Incubate at room temperature for a proper color development (5-15 minutes). pinik-ONE TMB produces a deep blue color during the enzymatic degradation of H_2O_2 by peroxidase.
2) After sufficient color development, add 100 ul Stop Solution (2M H_2SO_4) to each well.

   NOTE: Please be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Please visually monitor the color development to optimize the incubation time.

   Note: According to the reaction intensity, the color changes to violet, then deep blue during a reaction.

3) Using a microtiter plate reader, read the plate at the wavelength that is appropriate for the substrate used (450 nm for TMB)

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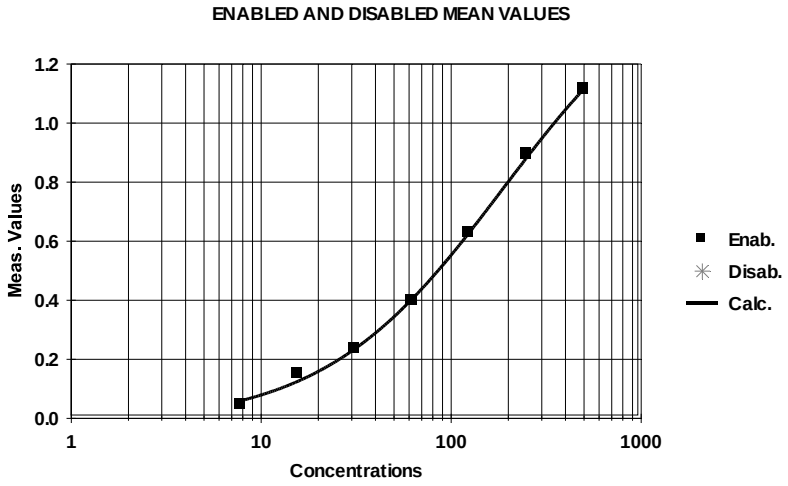
6. Calculation of Results

1) Average the duplicate readings from each standard, control, and sample.
2) Subtract the zero reading from each averaged value above.
3) Create a standard curve by reducing the data using ELISA reader's computer software capable of generating Standard curve-fit.

***** OD reading of standard should be generated for each set of samples (See example).



Pig IgG (ng/ml)
(15 minutes color development)

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11. Trouble shooting

Problem	Probable Cause	Solution
Low O.D.	Reagents not fresh or not at the correct pH	Ensure reagents have been prepared correctly and are in date.
	Incubation time not long enough	Ensure you are incubating the antibody for the recommended amount of time, if an incubation time is suggested.
	Incubation temperature too low	incubators are set at the correct temperature and working. Ensure all reagents are at room temperature before proceeding.
	Stop solution not added	Addition of stop solution
High O.D.	Standard reconstituted with less volume than required	Reconstitute lyophilized standard with correct volume of solution recommended in the protocol.
	Detection antibody, Streptavidin-HRP, Substrate solutions incubation times are too long	Decrease incubation time.
Poor Duplicates	Multichannel pipette errors	Calibrate the pipettes.
	Plate washing was not adequate or uniform	Make sure pipette tips are tightly secured. Confirm all reagents are removed completely in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before pipetting.
	Samples may have high particulate matter	Remove the particulate matter by centrifugation.
	Cross-well contamination	Do not use used plate sealers Do not use used pipette tips
High background	Contamination of reagents/samples	Use fresh reagents and pipette carefully.
	Insufficient washing of plates	Ensure well areas are washed adequately by filling the wells with wash buffer.
	Too much antibody used leading to non-specific binding	Try using less antibody.
	HRP conjugate to strong or left on too long	Check dilution of conjugate, use it at the recommended dilution.
	Substrate solution or stop solution is not fresh	Use fresh substrate solution.
	Plate left too long before reading on the plate reader	Color will keep developing (though at a slower rate if stop solution has been added).
	Contaminants from labware	Ensure reagents are fresh and prepared in clean labware.
	Incubation temperature too high	incubators are set at the correct temperature and working.
	Non-specific binding of antibody	2. Incorrect dilutions or pipetting errors
Sample readings are out of range	Samples contain no or below detectable levels of analyte or Samples contain analyte concentrations greater than highest standard point.	If samples are below detectable levels, it may be possible to use higher sample volume. If samples are high detectable levels, it may may require dilution and reanalysis.

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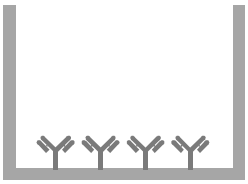
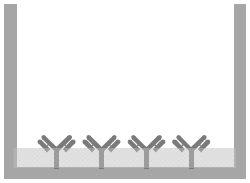
Summary of the ELISA procedure

: Capture Antibody
: Antigen
: Detection Antibody - HRP



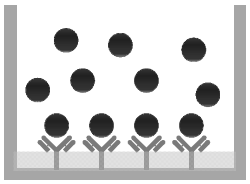
Coat the Plate with capture antibody and incubate for overnight at 4°C

Wash the plate 4 times with PBST



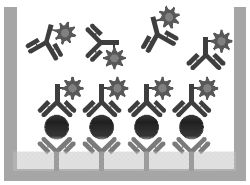
Block the plate with blocking reagent and incubate for 1 hr at room temperature.

Wash the plate 4 times with PBST



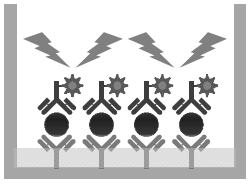
Add Standard or Samples and incubate for 1 hr at room temperature.

Wash the plate 4 times with PBST



Add Detection Antibody and incubate for 1 hr at room temperature

Wash the plate 4 times with PBST



Color development with TMB substrate and read the plate at 450 nm wavelength

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Related Products : KOMA Immunoglobulin ELISA Kit, pink-ONE

Cat. No.	Description
K3231006P	Albumin, Human, ELISA Complete Kit, pink-ONE
K3231007P	Albumin, Mouse, ELISA Complete Kit, pink-ONE
K3231008P	Albumin, Pig, ELISA Complete Kit, pink-ONE
K3231131P	Albumin, Rat , ELISA Complete Kit, pink-ONE
K3231012P	IgA, Bovine, ELISA Complete Kit, pink-ONE
K3231034P	IgA, Chicken, ELISA Complete Kit, pink-ONE
K3231064P	IgA, Human, ELISA Complete Kit, pink-ONE
K3231081P	IgA, Mouse, ELISA Complete Kit, pink-ONE
K3231092P	IgA, Pig, ELISA Complete Kit, pink-ONE
K3231104P	IgA, Rat, ELISA Complete Kit, pink-ONE
K3231066P	IgE, Human, ELISA Complete Kit, pink-ONE
K3231082P	IgE, Mouse, ELISA Complete Kit, pink-ONE
K3231126P	IgE, Rat, ELISA Complete Kit, pink-ONE
K3231014P	IgG, Bovine, ELISA Complete Kit, pink-ONE
K3231089P	IgG, Chicken, ELISA Complete Kit, pink-ONE
K3231067P	IgG, Human, ELISA Complete Kit, pink-ONE
K3231083P	IgG, Mouse, ELISA Complete Kit, pink-ONE
K3231094P	IgG, Pig, ELISA Complete Kit, pink-ONE
K3231009P	IgG, Rat, ELISA Complete Kit, pink-ONE
K3231020P	IgM, Bovine, ELISA Complete Kit, pink-ONE
K3231033P	IgM, Chicken, ELISA Complete Kit, pink-ONE
K3231069P	IgM, Human, ELISA Complete Kit, pink-ONE
K3231088P	IgM, Mouse, ELISA Complete Kit, pink-ONE
K3231096P	IgM, Pig, ELISA Complete Kit, pink-ONE
K3231110P	IgM, Rat, ELISA Complete Kit, pink-ONE

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