PROMPT
Proteins for Research

ISO 9001:2015
ISO 13485:2016

Dog PG (Progesterone) ELISA Kit

Cat: OPK0622

For research use only. Not intended for diagnostic use.

Detection Range: 78.13-5000pg/mL

Sensitivity: 46.88pg/mL

Test Principle

This ELISA kit uses the Competitive-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with Dog PG. During the reaction, Dog PG in the sample or standard competes with a fixed amount of Dog PG on the solid phase supporter for sites on the Biotinylated Detection Ab specific to Dog PG. Excess conjugate and unbound sample or standard are washed away, and Avidin-Horseradish Peroxidase (HRP) conjugate are added to each micro plate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns from blue to yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The concentration of Dog PG in tested samples can be calculated by comparing the OD of the samples to the standard curve.

Kit Components

An unopened kit can be stored at 2-8℃ for 6 months. If the opened kit is not used up, store the items separately according to the following conditions.

Reagents	Quantity		Storage Condition
	96T	48T	
Micro ELISA Plate	12 strips x 8 wells	6 strips x 8 wells	4℃ /-20℃ (12 months)
Reference Standard	2vials	1vials	4℃ /-20℃ (12 months)
Concentrated Biotinylated Detection Ab (100×)	60μL	60μL	4℃ /-20℃ (12 months)
Concentrated HRP Conjugate (100×)	120μL	60μL	4℃ /-20℃ (12 months)
Reference Standard & Sample Diluent	20mL	20mL	4℃ (12 months)
Biotinylated Detection Ab Diluent	13mL	13mL	4℃ (12 months)
HRP Conjugate Diluent	13mL	13mL	4℃ (12 months)
Concentrated Wash Buffer (25×)	30mL	30mL	4℃ (12 months)
Substrate Reagent	10mL	10mL	4℃ (12 month, store in dark)
Stop Solution	10mL	10mL	4℃ (12 months)
Plate Sealer	5 pieces	5 pieces	RT

Storage and Stability

1. All reagents should be stored as indicated on the component label.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25℃). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Materials Required, Not Supplied

1. Microplate reader with 450 nm wavelength filter (preheat it for 15 minutes before OD measurement)
2. High-precision transfer pipette, EP tubes and disposable pipette tips
3. Incubator capable of maintaining 37℃
4. Deionized or distilled water
5. Absorbent paper
6. Loading slot

Safety Notes

1. Please wear lab coats, goggles and latex gloves for protection. Please perform the experiment following the national security protocols of biological laboratories, especially when detecting blood samples or other bodily fluids.
2. A freshly opened ELISA plate may appear a water like substance, which is normal and will not have any impact on the experimental results. Return the unused wells to the foil pouch and store according to the conditions suggested in the above table.
3. Do not reuse the reconstituted standard, biotinylated detection Ab working solution, concentrated HRP conjugate working solution. The unspent undiluted concentrated biotinylated detection Ab (100×) and other stock solutions should be stored according to the storage conditions in the table.
   Do not use expired reagents. If using the kit in multiple sessions, prepare each component according to the required amount to avoid waste and ensure proper use in subsequent experiments. It is recommended to keep the microplate frame, return any unused wells to the aluminum foil bag, and ensure that all components are used within their shelf life.
4. Do not mix or substitute reagents from other batches or sources with those provided in this kit.
5. Do not reuse diluted standards, biotinylated antibody working solutions, or enzyme conjugate working solutions. The highest concentration standard working solution can be stored at -20℃ for up to two weeks, but should not be freeze-thawed repeatedly.
6. It is recommended that all samples and standards be assayed in duplicate.

Sample Collection and Storage

Serum - Samples should be collected into a serum separator tube. After clotting for 2 hours at room temperature or overnight at 4℃, and then centrifuging at 1000×g for 20 minutes. Assay freshly prepared serum immediately or store samples in aliquot at -20℃ or -80℃ for later use. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples at 1000×g and 2- 8℃ for 15 minutes within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20℃ or -80℃ for later use. Avoid repeated freeze-thaw cycles.

Tissue homogenates -The preparation of tissue homogenates will vary depending upon tissue type.

1. Rinse the tissues in pre-cooled PBS to completely remove excess blood, and weigh them before homogenization.

2. Mince the tissues to small pieces and homogenized them in fresh lysis buffer (different lysis buffer needs to be chosen based on subcellular location of the target protein)(PBS can be used as the lysis buffer of most tissues) (w:v=1:9, e.g. 900μL lysis buffer is added in 100 mg tissue sample) with a glass homogenizer on ice (micro tissue grinders, too).
3. Ultrasound the obtained suspension with an ultrasonic cell disrupter until the solution is clear.
4. Then, centrifuge the homogenates for 5 minutes at 10000×g and collect the supernatant and assay immediately or store in aliquots at ≤ -20℃.

Note: Tissue homogenates are recommended to be tested for protein concentration at the same time to obtain a more accurate concentration of the test substance per mg of protein.

Cell lysates - Cells need to be lysed before assaying according to the following directions.

1. Adherent cells should be washed by pre-cooled PBS gently, and then be detached with trypsin, and collect them by centrifugation at 1000×g for 5 minutes (suspension cells can be collected by centrifugation directly).
2. Wash cells 3 times in pre-cooled PBS.
3. Then, resuspend the cells in fresh lysis buffer with concentration of 10^7 cells/mL. If it is necessary, the cells could be subjected to ultrasonication until the solution is clear.
4. Centrifuge at 1500 ×g for 10 minutes at 2-8℃ to remove cellular debris. Assay immediately or store in aliquots at ≤-20℃.

Urine - Collect the first urine of the day (mid-stream) and discharge it directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤-20℃.Avoid repeated freeze-thaw cycles.

Saliva - Collect saliva using a collection device or equivalent. Centrifuge samples at 1000×g at 2-8℃ for 15 minutes. Remove particulates and assay immediately or store samples in aliquot at ≤-20℃. Avoid repeated freeze-thaw cycles.

Feces- Dry feces were collected as much as possible, weighing more than 50 mg. The feces were washed three times with PBS (w:v = 1:9, e.g. 900μL lysis buffer is added in 100 mg feces), sonicated (or mashed) and centrifuged at 5000×g for 10 minutes, where the supernatant was collected for testing.

Cell culture supernatants and other biological fluids - Centrifuge samples at 1000×g for 20 minutes. Collect the supernatant and assay immediately or store samples in aliquot at -20℃ or -80℃ for later use. Avoid repeated freeze-thaw cycles.

Notes

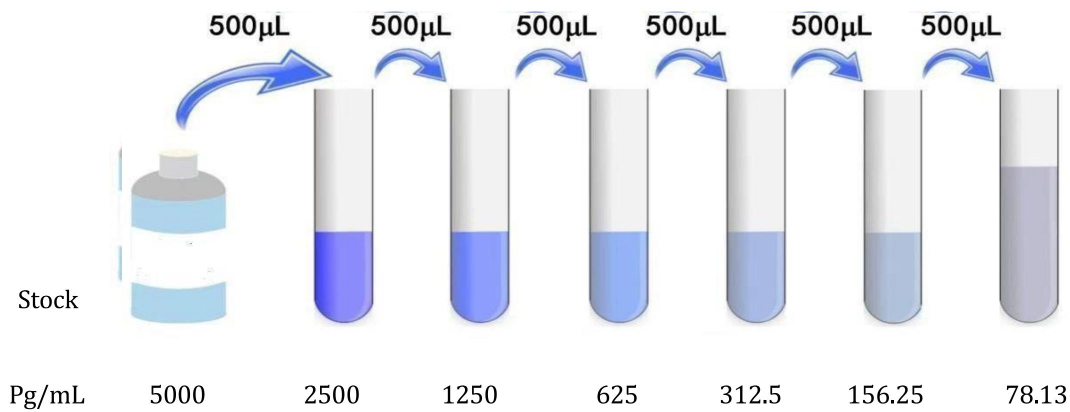
1. Samples to be used within 5 days may be stored at 4℃, otherwise samples must be stored at -20℃ (≤1 month) or -80℃ (≤2 months) to avoid loss of bioactivity and contamination. Avoid repeated freeze-thaw cycles.
2. Sample hemolysis will influence the result, so it should not be used.
3. When performing the assay, bring samples to room temperature.

Reagent Preparation

1. Bring all reagents to room temperature (18-25℃) before If the kit will not be used up in one assay, please only take out the necessary strips and reagents for present experiment, and store the remaining strips and reagents at required condition.

2. **Wash Buffer:** Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40 ℃ water bath and mix it gently until the crystals have completely dissolved.

3. **Standard working solution:**
   a. Centrifuge the standard at 10, 000×g for 1 minutes.
   b. Add 1mL of Reference Standard & Sample Diluent, let it stand for 10 minutes and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 5000pg/mL.
   c. Take 7 EP tubes, add 500μL standard & sample diluent to each tube, take 500μL from 5000pg/mL to make 2500pg/mL, and so on, the last tube is blank (no more liquid needs to be drawn from the penultimate tube.) Use it now.



4. **Biotinylated Detection Ab working solution:** Calculate the required amount before the experiment (50µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated Biotinylated Detection Ab at 800×g for 1 minutes, then dilute the 100× Concentrated Biotinylated Detection Ab to 1× working solution with Biotinylated Detection Ab Diluent.

5. **HRP Conjugate working solution:** Calculate the required amount before the experiment (100µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated HRP Conjugate at 800×g for 1 minutes, then dilute the 100× Concentrated HRP Conjugate to 1× working solution with HRP Conjugate Diluent.

6. Experimental Operation Tips

a) Solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.

b) Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.

c) After adding the Substrate Reagent. The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes.

d) Adding the stop solution should be done in the same order as the substrate solution.

Assay Protocol

1. Determine wells for diluted standard, blank and sample. Add 50µL each dilution of standard, blank and sample into the appropriate wells.
2. Immediately add 50µL of Biotinylated Detection Ab working solution to each well. Cover the plate with a new sealer. Incubate for 30 minutes at 37°C.
3. Decant the solution from each well add 300µL of wash buffer to each well. Soak for 0.5 minutes and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times.
4. Add 100µL of HRP Conjugate working solution to each well. Cover the plate with a new sealer. Incubate for 30 minutes at 37°C.
5. Decant the solution from each well, repeat the wash process for 3 times as conducted in step 3.
6. Add 100µL of Substrate Reagent to each well. Cover the plate with a new sealer. Incubate for about 15 minutes at 37°C. Protect the plate from light.
7. Add 50µL of Stop Solution to each well.
8. Determine the optical density (OD value) of each well at once with a micro plate reader set to 450 nm.

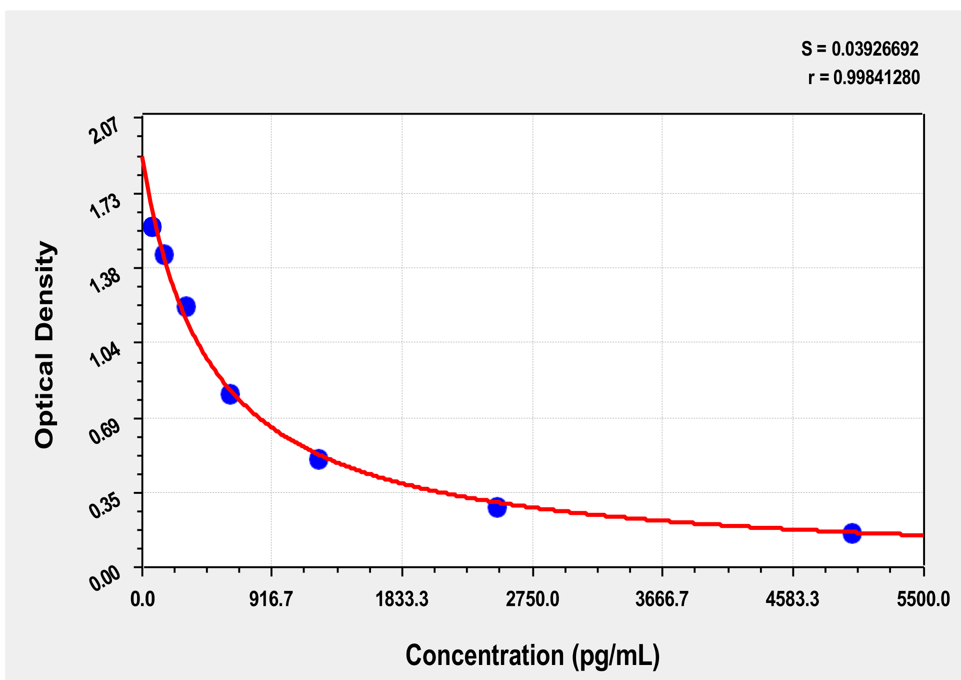
Calculation of Results

I. The reference standard curve in this manual is for guidance only. The experimenter should use the standard data from each experiment to construct the standard curve and calculate the concentration of the target substance in the sample.

II. When constructing the standard curve, first calculate the average OD values of the standards and sample replicates. Then, plot the concentration on the X-axis and the corrected OD values on the Y-axis, and use software to perform a nonlinear four-parameter logistic (4PL) fit. This will provide the standard curve, from which the sample concentration can be calculated.

III. The actual concentration is the calculated concentration multiplied by the dilution factor.

Concentration (pg/mL)	Corrected OD
5000	0.156
2500	0.275
1250	0.499
625	0.801
312.5	1.204
156.25	1.442
78.13	1.569
0	1.911



Sensitivity

The minimum detection concentration of the Dog PG detected by this kit is usually less than the 46.88pg/mL. The sensitivity was determined by adding two standard deviations to the mean OD value of twenty zero standard replicates and calculating the corresponding concentration.

Origin

Precision

Mean coefficient of variation for Intra- Assay and Inter-Assay: 3 samples with low, middle and high level concentration were tested for repeat multiple times, respectively. The results showed that the coefficient of variation of the kits was less than 10%, which met the precision quality control standard.

Specificity

This method has high sensitivity and specificity for the detection of Dog PG. No significant cross- reactivity was observed between the Dog PG and its analogues.

Recovery

The recovery rate of this kit is 80-120%, meeting the recovery rate quality control standard. The recovery rate is divided into spiked recovery and sample dilution linear recovery. Spiked Recovery: Known concentrations of Dog PG are added to different samples for recovery experiments, resulting in the recovery rate range and average value. Sample Dilution Linear Recovery: Samples containing Dog PG are diluted at different ratios (e.g., 2x, 4x, etc.), and the recovery rate range and average value are Determined.

Linearity

Dilute linear experiments were performed on samples with Dog PG to evaluate the linearity of the kit. The results showed that the kit linear range (%) was 80-120%, which met the linear quality control standard.

Declaration

I. Limited by current conditions and scientific technology, we can't conduct comprehensive identification and analysis of all the raw material provided. There might be some qualitative and technical risks for users using the kit.

II. This assay is designed to eliminate interference by factors present in biological samples. Until all factors have been tested in the ELISA immunoassay, the possibility of interference cannot be excluded.

III. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions.

IV. Incorrect results may occur because of incorrect operations during the reagents preparation and loading, as well as incorrect parameter settings of the Micro plate reader. Please read the instructions carefully and adjust the instrument prior to the experiment.

V. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.

VI. Every kit has strictly passed QC test. However, results from end users might be inconsistent with our data due to some variables such as transportation conditions, different lab equipment, and so on. Intra assay variance among kits from different batches might arise from the above reasons too.

VII. Kits from different manufacturers or other methods for testing the same analyte could bring out inconsistent results, since we haven't compared our products with those from other manufacturers.

VIII. The kit is designed for research use only, we will not be responsible for any issues if the kit is applied in clinical diagnosis or any other related procedures.

Analysis of Common Problems and Causes of ELISA Experiment

High background/Non-specific staining

Description of results	Possible reason	Recommendations and precautions
After termination, the whole plate results show a uniform yellow or light colour; or the Standard curve is linear but the background is too high	The yellowing of the whole plate may be caused by wrong addition of other reagents	Check the components and lot numbers of the reagents before the experiment, and confirm that all components belong to the corresponding kit. Reagents from different kits or different lot numbers cannot be mixed.
	ELISA plate was not washed sufficiently	Make sure that the same amount of Washing Solution is added to each microwell during the washing process. After washing, press the ELISA plate firmly on the absorbent paper to remove the residual buffer.
	Incubation time too long	Please strictly follow the steps of the manual
	Streptavidin-HRP contaminates the tip and TMB container or positive control contaminates the Pre-coated Microplate	When absorbing different reagents, the tips should be replaced. When configuring different reagent components, different storage vessels should be used. Please use a pipette during operation.
	Biotinylated-Conjugate or Streptavidin-HRP concentration too high	Check whether the concentration calculation is correct or use after further dilution.
	Substrate exposure or contamination prior to use	Store in the dark at all times before adding substrate.
	Colour development time is too long	Please strictly follow the steps of the manual.
	The wrong filter was used when the absorbance value was read	When TMB is used as the substrate, the absorbance should be read at 450 nm.

No colour plate

Description of results	Possible reason	Recommendations and precautions
After the colour development step, all wells of the ELISA plate are colourless; the positive control is not obvious	Mixed use of component reagents	Please read labels clearly when preparing or using
	In the process of plate washing and sample addition, the enzyme marker is contaminated and inactivated, and loses its ability to catalyze the colour developing agent	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN_3, etc.), and confirm that the container for preparing the Wash Solution has been washed.
	Missing a reagent or a step	Review the manual in detail and strictly follow the operating steps

Light colour

Description of results	Possible reason	Recommendations and precautions
The Standard is normal, the colour of the sample is light	The sample uses NaN_3 preservative, which inhibits the reaction of the enzyme	Samples cannot use NaN_3
	The sample to be tested may The standard is normal, the colour of the sample is lightnot contain strong positive samples, so the result may be normal	In case of doubt, please test again.
The visual result is normal, but the reading value of the microplate reader is low	Wrong filter used for absorbance reading	When TMB is used as the substrate, the absorbance should be read at 450 nm.

Description of results	Possible reason	Recommendations and precautions
	Insufficient incubation time	Timer accurate timing
	Insufficient colour reaction	Usually 15 - 30 minutes
	The number of washings increases, and the dilution ratio of the concentrated lotion does not meet the requirements	Reduce the impact of washing, dilute the concentrated lotion and washing time according to the manual, and accurately record the washing times and dosage.
	Distilled water quality problem	The prepared lotion must be tested to see if the pH value is neutral.

<table>
<tr><td rowspan="4">All wells, including Standard and Samples, are lighter in colour</td><td>In the process of plate washing and sample addition, the enzyme marker is contaminated and inactivated, and loses its ability to catalyze the colour developing agent.</td><td>Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN_3, etc.), confirm that the container for preparing the Washing Solution has been washed, and confirm that the purified water for preparing the Washing Solution meets the requirements and is not contaminated.</td></tr>
<tr><td>The kit has expired or been improperly stored</td><td>Please use it within the expiration and store it in accordance with the storage conditions recommended in the manual to avoid contamination.</td></tr>
<tr><td>Reagents and samples are not equilibrated before use</td><td>All reagents and samples should be equilibrated at room temperature for about 30 minutes.</td></tr>
<tr><td>Insufficient suction volume of the pipette, too fast discharge of pipetting suction, too much liquid hanging on the inner wall of the tip or the inner wall is not clean.</td><td>To calibrate the pipette, the tips should be matched, each time the tips should fit tightly, the pipetting should not be too fast, and the discharge should be complete. The inner wall of the tips should be clean, and it is best to use it once.</td></tr>
</table>

<table>
<tr><th>Description of results</th><th>Possible reason</th><th>Recommendations and precautions</th></tr>
<tr><td rowspan="6">Poor repeatability</td><td>Incubation temperature constant temperature effect is not good</td><td>Keep the temperature constant to avoid the local temperature being too high or too low</td></tr>
<tr><td>When adding liquid, too much remains on the medial wall of wells</td><td>When adding liquid, the tip should try to add liquid along the bottom of the medial wall of wells without touching the bottom of the hole.</td></tr>
<tr><td>Reuse of consumables</td><td>The tips should be replaced when different reagents are drawn, and different storage vessels should be used when configuring different reagent components.</td></tr>
<tr><td rowspan="2">The bottom of the microwell is scratched or there is dirt</td><td>Be careful when operating, be careful not to touch the bottom and wipe the bottom of the microplate to remove dirt or fingerprints.</td></tr>
<tr><td>Technical repetition of the same sample for 3 times, including more than 2 approximate values.</td></tr>
<tr><td>Cross-contamination during sample addition</td><td>Try to avoid cross-contamination when adding samples</td></tr>
</table>

The colour of plate is chaotic and irregular	Cross-contamination from manual plate washing	When washing the plates by hand, the first 3 injections of the lotion should be discarded immediately, and the soaking time should be set for the next few times to reduce cross-contamination.
	Cross-contamination when clapping	Use a suitable absorbent paper towel when clapping the plate, do not pat irrelevant substances into the well of the plate, and try not to pat in the same position to avoid cross-contamination.

Description of results	Possible reason	Recommendations and precautions
The colour of plate is chaotic and irregular	The liquid filling head of the plate washer is blocked, resulting in unsatisfactory liquid addition or large residual amount of liquid suction, resulting in the colour of plate is chaotic and irregular	Unblock the liquid addition head, so that each well is filled with washing liquid when washing the plate and the residual amount should be small when aspirating liquid.
	Incomplete centrifugation of the sample, resulting in coagulation in the reaction well or interference of sediment or residual cellular components	Serum plasma should be fully centrifuged at 3000 rpm for more than 6 minutes
	The sample is stored for too long time, resulting in contamination.	Samples should be kept fresh or stored at low temperature to prevent contamination
	Incorrect preparation of Washing Solution or direct misuse of concentrated Washing Solution	Please configure according to the manual