(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSTICS !)

Catalog No: E-OSEL-C0001

Product size: 96T/48T/24T/96T*5

Elabscience® QuicKey Pro Canine E2 (Estradiol) ELISA Kit

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help (info in the header of each page).

Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please refer to specific expiry date from label outside of box.

Please kindly provide us with the lot number (on the outside of the box) of the kit for more efficient service.

Rev V5.0

QuicKey Pro™ Series

Get more sensitive and precise results with saving at least 1-2h comparing to traditional ELISA Kits. The new developed technology in house will help to accelerate your science research in a more efficient way.

Intended Use

Designed for the *in vitro* quantitative determination of Canine Estradiol (E2) concentrations in serum, plasma and other biological fluids.

Performance Characteristics

Parameter	Specification
Sensitivity	6.37 pg/mL
Detection Range	15.63-1000 pg/mL
Specificity	Specific for Canine E2. No significant cross-reactivity or interference with related analogues was observed
Repeatability	Coefficient of variation is < 10%

Test Principle

This ELISA kit uses the Competitive-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with Canine E2. Samples (or Standards) and Horseradish Peroxidase (HRP) linked antibody specific for Canine E2 are added to the micro ELISA plate wells. Canine E2 in samples (or standards) competes with a fixed amount of E2 on the solid phase supporter for sites on the HRP linked detection antibody specific to E2. Excess conjugate and unbound sample or standard are washed from the plate.

Upon reaction with a chromogenic substrate, the HRP enzyme catalyzes the development of a colored product. The resulting signal intensity, measured spectrophotometrically at 450 ± 2 nm, is inversely proportional to the concentration of Canine E2 in the sample. Quantitative determination is achieved by comparison with a standard curve generated from known concentrations.

Kit Components & Storage

An unopened kit should be stored at 2-8°C for 6 months. After opening, components should be stored under the conditions specified below:

Item	Specifications	Storage After Preparation
Micro ELISA Plate (Dismountable)	96T: 8 wells ×12 strips 48T: 8 wells ×6 strips 24T: 8 wells ×3 strips 96T*5: 5 plates, 96T	2-8 ℃, 1 month
Reference Standard	96T: 2 vials 48T/24T: 1 vial 96T*5: 10 vials	2-8 ℃, use the reconstituted standard within 24h
Concentrated HRP Conjugate (100×)	96T: 1 vial, 60 µL 48T/24T: 1 vial, 30 µL 96T*5: 5 vials, 60 µL	2-8 ℃ (Protect from light)
Reference Standard & Sample Diluent	96T/48T/24T: 2 vials, 20 mL 96T*5: 10 vials, 20 mL	2-8 ℃
HRP Conjugate Diluent	96T/48T/24T: 1 vial, 14 mL 96T*5: 5 vials, 14 mL	
Concentrated Wash Buffer(25×)	96T/48T/24T: 1 vial, 30 mL 96T*5: 5 vials, 30 mL	
Substrate Reagent	96T/48T/24T: 1 vial, 10 mL 96T*5: 5 vials, 10 mL	2-8 ℃ (Protect from light)
Stop Solution	96T/48T/24T: 1 vial, 10 mL 96T*5: 5 vials, 10 mL	2-8 ℃
Plate Sealer	96T/48T/24T: 5 pieces 96T*5: 25 pieces	
Manual	1 copy	

Note

Reagents may contain slightly more volume than indicated on the label; mix and centrifuge the vial of reagents before use. Use precise measuring instruments rather than pouring directly from the vial.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm
- High-precision pipettes, EP tubes, and disposable pipette tips
- Incubator capable of maintaining 37 °C
- Deionized or distilled water
- Absorbent paper
- Loading slot (Suction Tank)

Precautions

1. **For Research Use Only**
   - This kit is intended for research purposes and is not for diagnostic or therapeutic use.
2. **Laboratory Safety**
   - Always wear lab coats, safety goggles, and disposable gloves when handling reagents or biological samples.
   - Follow local and national biosafety regulations, especially when working with blood, plasma, or other bodily fluids.
3. **Reagent Handling**
   - Protect light-sensitive reagents such as **Concentrated HRP Conjugate (100×)** and **Substrate Reagent** from exposure to light.
   - Ensure all reagent caps are tightly closed to prevent evaporation and microbial contamination.
   - Do **not reuse** reconstituted standards, working solutions of detection antibody, or HRP conjugate. Stock solutions must be stored according to specified conditions.

- Do not mix reagents from different kit lots or sources.

4. **Pipetting and Cross-Contamination Prevention**
   - Use fresh tips for each standard, sample, and reagent addition.
   - Use separate reservoirs for each reagent to prevent cross-contamination.
5. **Kit Expiry**
   - Do not use the kit beyond the expiration date indicated on the label.

Sample Collection & Handling

Proper sample handling and collection are critical for the accurate measurement of Canine E2. The following guidelines are recommended:

General Guidelines

- Use disposable, endotoxin-free tubes for blood collection.
- Avoid samples with high hemolysis or excessive lipids, as they may interfere with the assay.
- Predict sample concentration beforehand to determine appropriate dilutions.

Storage and Stability

- For longer storage: -20 °C for up to 1 month, −80 °C for up to 3 months.
- Avoid repeated freeze-thaw cycles. Thaw frozen samples slowly and centrifuge to remove precipitates before assay.

Sample Preparation Considerations

- When using lysis buffers for tissue homogenates or cell lysates, note that chemical components may affect assay results.
- If the sample type is not listed in the manual, perform preliminary validation experiments.
- Some recombinant proteins may not be detected due to mismatches with coated or detection antibodies.

Sample Types and Preparation Guidelines

Serum

- Collect blood in pyrogen-free, endotoxin-free coagulation tubes or plain tubes.
- Allow clotting, at room temperature, for 2 hours, or overnight, at 2-8 °C.
- Centrifuge at 1,000 × g for 20 minutes. For hyperlipidemic samples, centrifuge at 10,000 × g at 2-8 °C for 10 minutes to separate serum and remove chylomicrons.
- Aliquot the supernatant (serum) into sterile cryopreservation tubes.

Plasma

- Use EDTA-K2/Na2 (1.5-2 mg/mL) as anticoagulant; sodium citrate can be used for coagulation-related targets.
- Gently invert tubes 5-8 times immediately after collection to prevent air bubbles and hemolysis.

- Centrifuge at 1,000 × g at 2-8 °C for 15 minutes, within 30 minutes after collection.
- Collect the plasma fraction after centrifugation.

Saliva

- Remove particulates by centrifugation for 10 minutes at 4000×g at 2-9°C. Collect the supernatant to carry out the assay. Recommend to use fresh saliva samples.

Urine

- Centrifuge at 1,500 × g at 2-8 °C for 15 minutes to remove particulate matter.
- Use supernatant immediately or aliquot and store at ≤ -20 °C.

Recommended Reagents for Sample Processing

- PMSF Protease Inhibitor (Cat No. E-EL-SR002)
- 0.25% Trypsin Solution (Cat No. E-EL-SR001)
- BCA Protein Quantification Kit (Cat No. E-BC-K318-M)

Dilution method

- Estimate the expected concentration of your samples before the assay.
- It is recommended to do the experiment with undiluted Canine serum, plasma samples.
- Use the same diluent provided in the kit to maintain assay consistency.

Sample Dilution Protocol for Dilution Factors > 100-Fold

The following is the general procedure that can be adjusted based on sample volume and dilution factor:

General Principles

- Serial dilution is recommended; the dilution factor at each step should not exceed 100-fold.

  Pipetting volume at each step should be ≥ 3 µL. Mix thoroughly and avoid air bubbles.

Procedure Examples

100-fold dilution: One-step dilution. Add 5 µL sample to 495 µL sample diluent.

1,000-fold dilution: Two-step dilution. Add 5 µL sample to 95 µL sample (20-fold), then 5 µL 20-fold diluted sample to 245 µL sample diluent (final 1,000-fold).

10,000-fold dilution: Two-step dilution. Add 5 µL sample to 495 µL sample diluent (100-fold), then 5 µL 100-fold diluted sample to 495 µL sample diluent (final 10,000-fold).

100,000-fold dilution: Three-step dilution. Add 5 µL sample to 195 µL sample diluent (40-fold), then 5 µL 40-fold diluted sample to 245 µL sample diluent (50-fold dilution), and finally 5 µL 2,000-fold diluted sample to 245 µL sample diluent (final: 100,000-fold).

Higher dilution factors can be achieved by increasing the number of serial dilutions, following the same principles. Select the dilution strategy based on the sample volume and estimated concentration to ensure accuracy and repeatability.

Reagent preparation

1. General Handling

- Equilibrate all reagents to room temperature (18-25°C) before use.
- If the kit is to be used partially in a single assay, take only the strips and reagents required for the current assay, leaving the remainder under the conditions specified in the storage table.

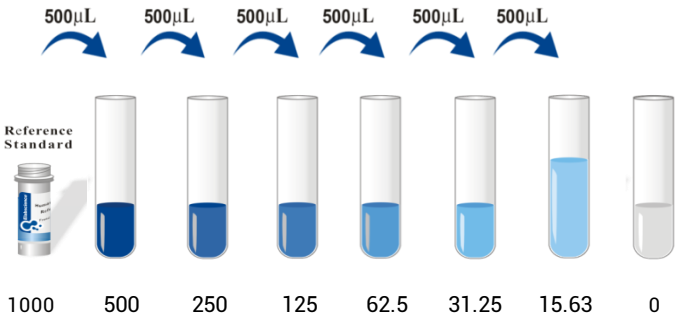
2. **Wash Buffer**

- Prepare the working Wash Buffer by diluting 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water yielding 750 mL.
- Note: If crystalline precipitates have formed in the concentrate, warm it gently in a 40 °C water bath and mix until fully dissolved. Use the prepared Wash Buffer on the same day only.

3. **Standard Working Solution**

- Centrifuge the standard at 10,000 × g for 1 minute.
- Reconstitute with 1 mL of the Standard & Sample Diluent, allow to stand for 10 minutes, and gently invert several times until completely dissolved. Alternatively, vortex briefly at low speed; remove bubbles by low-speed centrifugation if necessary.
- This produces a 1000 pg/mL stock solution. Prepare serial dilutions according to experimental requirements. Recommended dilution gradient: 1000, 500, 250, 125, 62.5, 31.25, 15.63, 0 pg/mL.

- Serial Dilution Procedure:
    i. Dispense 500 µL of Standard & Sample Diluent into 7 EP tubes, respectively.
    ii. Transfer 500 µL of the 1000 pg/mL stock solution into the first tube to obtain 500 pg/mL.
    iii. Sequentially transfer 500 µL from the preceding tube into the next tube for subsequent dilutions. The illustration on the next page is provided for reference. Designate the last tube as the blank; do not transfer any solution into it.
- Aliquot the 1000 pg/mL stock and store at −20 °C. Use within two weeks and avoid repeated freeze-thaw cycles.
- Serially diluted standards should be prepared immediately, prior to use.



4. **HRP Conjugate Working solution:**

- Calculate the required volume (50 µL/well) plus a slight excess.
- Centrifuge the Concentrated HRP Conjugate (100×) at 800 × g for 1 minute.
- Dilute to 1× with HRP Conjugate Diluent at a ratio of 1:99.
- Prepare the working solution immediately, prior to use.

Assay procedure

1. **Sample, Standard Loading and HRP Conjugate Antibody**

- Assign wells for diluted standards, blanks, and samples.
- Add 50 µL of each standard, blank, or sample to the designated wells. Analyze all samples and standards in duplicate. Determine sample dilution factors based on preliminary experiments or technical support recommendations.
- Add 50 µL of HRP Conjugate Working Solution to each well immediately. Cover with a fresh sealer and incubate for 60 minutes at 37°C.
- Note: Dispense solutions gently to the bottom of the well, avoiding contact with the sidewalls and foaming.

2. **Washing Step 1**

- Decant the solution and add 350 µL of Wash Buffer to each well. Soak for 1 minute, then aspirate or decant and blot dry with clean absorbent paper.
- Repeat five times.
- Note: A microplate washer may be used. Do not allow wells to dry. Proceed immediately with the next step.

3. **Substrate Reaction**

- Add 90 µL of **Substrate Reagent** to each well. Cover and incubate for approximately 15 min at 37°C. Protect the plate from light.
- Note: Reaction time may be adjusted based on observed color development but should not exceed 30 minutes. Switch on the Microplate Reader for 15 min prior to OD measurement.

4. **Stopping the Reaction**

- Add 50 µL of **Stop Solution** to each well in the same order as the substrate addition.

5. **Optical Density Measurement**

- Measure the optical density (OD value) at 450 ± 2 nm using a micro-plate reader immediately after adding Stop Solution .

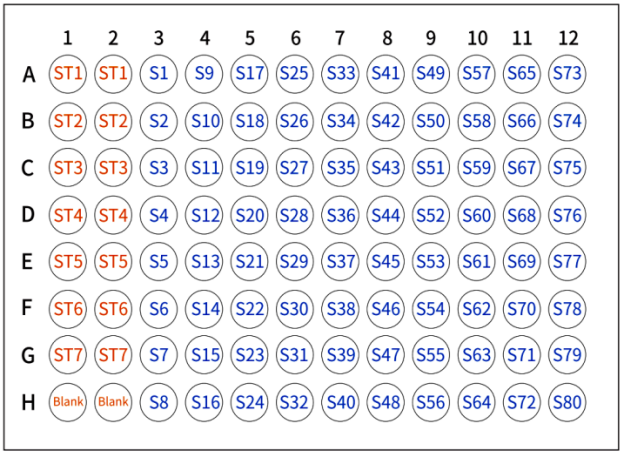
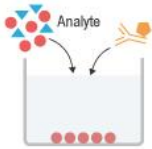


Figure: Example Plate Layout

ST: Standard curve well; Blank: Blank well (0 pg/mL).

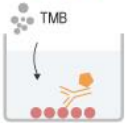
S: Sample well.

Assay Procedure Summary

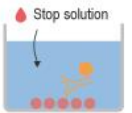


1. Add 50μL each standard and sample. Immediately add 50μL of HRP linked Ab working solution. Incubate for 60 min at 37℃.

2. Aspirate and wash the plate for 5 times



3. Add 90 μL of Substrate Reagent. Incubate for about 15 min at 37℃



4. Add 50μL of Stop Solution

5. Read the plate at 450nm immediately. Calculation of the results

Calculation of results

1. Data Processing

- Calculate the mean optical density (OD) of duplicate readings for each standard and sample.

2. Standard Curve

- Plot a four-parameter logistic (4PL) curve with standard concentrations on the x-axis (log scale optional) and OD values on the y-axis.

3. Sample Concentration

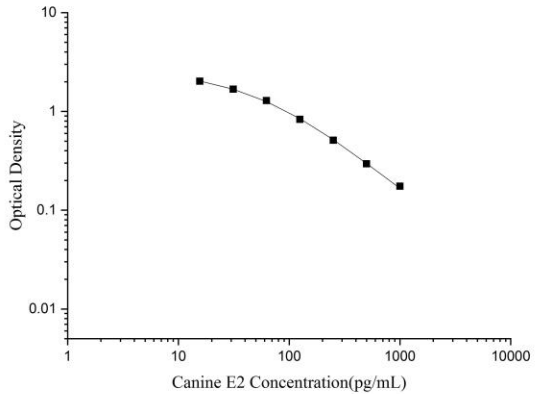
- Determine the concentration of each sample from the standard curve.
- If the sample OD falls below the lower limit of the standard curve, repeat the assay using an appropriate dilution.
- The final concentration is calculated using the following equation:

Final concentration = Measured concentration × Dilution factor

Typical data

The following data was generated by the Quality Control Department, under controlled laboratory conditions (ambient temperature: 18-25℃, relative humidity: 35-75%) using standardized procedures (TMB reaction at 37°C in the dark for 15 minutes, followed by termination and OD measurement). These values are provided for reference only. Actual results may vary due to differences in laboratory conditions, operator technique, and equipment. Users are required to generate a standard curve using their own experimental data.

pg/mL	OD1	OD2	Mean OD
1000	0.147	0.203	0.175
500	0.277	0.313	0.295
250	0.530	0.492	0.511
125	0.826	0.838	0.832
62.5	1.297	1.281	1.289
31.25	1.677	1.675	1.676
15.63	2.022	2.024	2.023
0	2.590	2.590	2.590



Sample Validation Data

This kit has been validated using samples from apparently healthy volunteers. The data is for reference only.

Sample type	Reference range of Canine E2 (pg/mL)
Serum(n=10)	25-64
Plasma(EDTA)(n=10)	27-59

Performance

■ Precision

Intra-assay Precision (Within-run Precision): Three samples representing low, mid, and high concentrations of Canine E2 were tested 20 times on a single plate.

Inter-assay Precision (Between-run Precision): Three samples representing low, mid, and high concentrations of Canine E2 were tested on three separate plates, with 20 replicates per plate, to assess variability among assays.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
Numbers	20	20	20	20	20	20
Mean (pg/mL)	45.48	142.49	489.6	49.01	155.25	478.12
Standard deviation	2.62	6.28	15.08	3.32	7.27	22.14
CV (%)	5.77	4.41	3.08	6.78	4.68	4.63

■ Recovery

The recovery of Canine E2 was evaluated by spiking samples at low, mid, and high concentrations across the assay range in various sample matrices. The assay performance was assessed by comparing the measured concentrations to the expected spiked amounts to determine the percent recovery.

Sample Type	Range (%)	Average Recovery (%)
Serum (n=8)	90-103	96
EDTA plasma (n=8)	85-99	92

■ Linearity

Linearity of the assay was evaluated by spiking samples with high concentrations of Canine E2 and performing serial dilutions using Standard & Sample Diluent to produce concentrations spanning the assay's dynamic range. The measured values were then compared to the expected concentrations to assess the linearity of response.

		Serum (n=5)	EDTA plasma (n=5)
1:2	Range (%)	100-112	88-101
	Average (%)	106	95
1:4	Range (%)	101-115	87-99
	Average (%)	108	94
1:8	Range (%)	98-117	85-99
	Average (%)	107	91
1:16	Range (%)	98-117	91-102
	Average (%)	106	97

■ Stability

Each kit batch is subjected to accelerated stability testing and real-time stability monitoring. Sample performance is evaluated after storage at 37 °C for 10 days to assess the impact of elevated temperature on assay reliability and reagent integrity.

	Variation range of 37°C mean concentration / 2-8°C mean concentration (%)
Sample 1 (n=16)	90.52-96.49
Sample 2 (n=16)	102.65-113.51

Declaration

1. Due to current technological and methodological limitations, comprehensive identification and analysis of all raw materials cannot be guaranteed. Users should be aware of potential qualitative and technical risks associated with kit use.
2. This assay is designed to minimize interference by factors present in biological samples; however, until all potential interfering substances are fully evaluated, their influence cannot be entirely excluded.
3. Experimental results are dependent on reagent quality, operator technique, and laboratory conditions. The manufacturer is responsible only for the performance of the kit itself, not for the samples utilized during the assay. Users should estimate and reserve sufficient sample volume for their experiments.
4. Optimal results are achieved only when all reagents supplied with the kit are used and the instructions are strictly followed.
5. Inaccurate results may occur due to improper reagent preparation, incorrect sample or reagent loading, or misconfigured microplate reader parameters. Users should carefully review instructions and calibrate instruments prior to assay.
6. Variability may occur even when the same operator performs separate assays. Consistent execution of all procedural steps is essential for reproducible results.
7. All kits undergo rigorous quality control; however, variations in results may arise from differences in transportation, laboratory equipment, or environmental conditions. Intra-assay variability may also occur between

different kit batches.

8. Results may differ when using kits from other manufacturers or alternative assay methods for the same analyte, as comparative evaluations have not been performed.
9. This kit is intended for research use only. The manufacturer is not responsible for any outcomes resulting from use in clinical diagnostics or other non-research applications.