Technical Manual

Canine TOX-IgG (Toxoplasma-Immunoglobulin G) ELISA Kit

- Catalogue Code: AEFI01684
- Qualitative ELISA Kit
- Research Use Only

Contents

1. Key features and Sample Types

Aliases:

TOX-IgG ELISA Kit, Toxoplasma-Immunoglobulin G ELISA Kit

Uniprot:

Not available

Detection method:

Qualitative ELISA

Sample Type:

Serum, Plasma, Cell culture supernatant, Cell or tissue lysate, Other liquid samples

Reactivity:

Canine

Storage:

2-8°C for 6 months (sealed), please do not freeze!

Expiry:

See Kit Label

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this unopened ELISA Kit at 4°C for 6 months. For opened kits, store individual components as described in the Kit Contents section. Date of expiration will be on the ELISA Box label.

3. Description and Principle

Assay Genie ELISA Kits are designed for the precise measurement of analytes in a wide variety of sample types. In response to the increasing demand for high-quality, consistent data from today's scientists, we have developed a range of sensitive, fast, and reliable ELISA kits that meet and exceed these expectations.

The Assay Genie Qualitative ELISA kit allows for the measurement of Canine TOX-IgG in Canine samples.

How do our ELISA kits work?

This kit is based on indirect enzyme-linked immune-sorbent assay technology. Canine TOX-IgG is pre-coated onto 96-well plates. The test samples were added to the wells, unbound conjugates were washed away with wash buffer. Then added HRP-conjugated antigen. TMB substrates were used to visualize HRP enzymatic reaction. It was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The optical density of developed color is read with a suitable photometer at 450nm with a selected reference wavelength within 650 nm.

4. Kit Contents

The sealed kit can be stored at 2-8 °C. The storage condition for opened kit is specified in the table below. Each kit contains reagents for either 48 or 96 assays including:

No.	Component	96-Well Kit	Storage
1	ELISA Microplate (dismountable)	8 x 12	Put the rest of the strips into a sealed foil bag with the desiccant. Store for 1 month at 2-8°C or for 6 months at -20°C
2	Positive Control	1ml x 1	2-8°C
3	Negative Control	1ml x 1	2-8°C
4	HRP- Conjugate Antigen	12ml x1	2-8°C
5	TMB substrate A	6ml x 1	2-8°C (Avoid Direct Light)
6	TMB substrate B	6ml x1	2-8°C (Avoid Direct Light)
7	Stop solution	6ml x1	2-8°C
8	Wash buffer (20X)	50ml x 1	2-8°C
9	Plate Sealer	3	
10	Manual	1	

Additional materials required:

1. 37°C incubator (CO2 incubator for cell culture is not recommenced).
2. Plate Reader with 450nm filter
3. Precision pipettes and disposable pipette tips
4. Distilled water
5. Clean tubes and Eppendorf tubes
6. Automated plate washer (optional)

Precautions:

1- To determine the concentration of your target, it's recommended to perform a pilot experiment using standards and a small number of samples.

2- After opening and before use, keep the plate dry.

3- Before using the kit, centrifuge tubes to spin down the standards and antibodies.

4- Store TMB reagents protected from light.

5- Proper washing steps are critical for assay success; any deviations may cause false positives and high background.

6- Use duplicate wells for both standard and sample testing.

7- Do not allow the microplate to dry during the assay, as dry plates can inactivate active components.

8- To avoid cross-contamination, do not reuse tips and tubes.

9- Avoid mixing reagents from different batches.

5. Sample Preparation

General considerations:

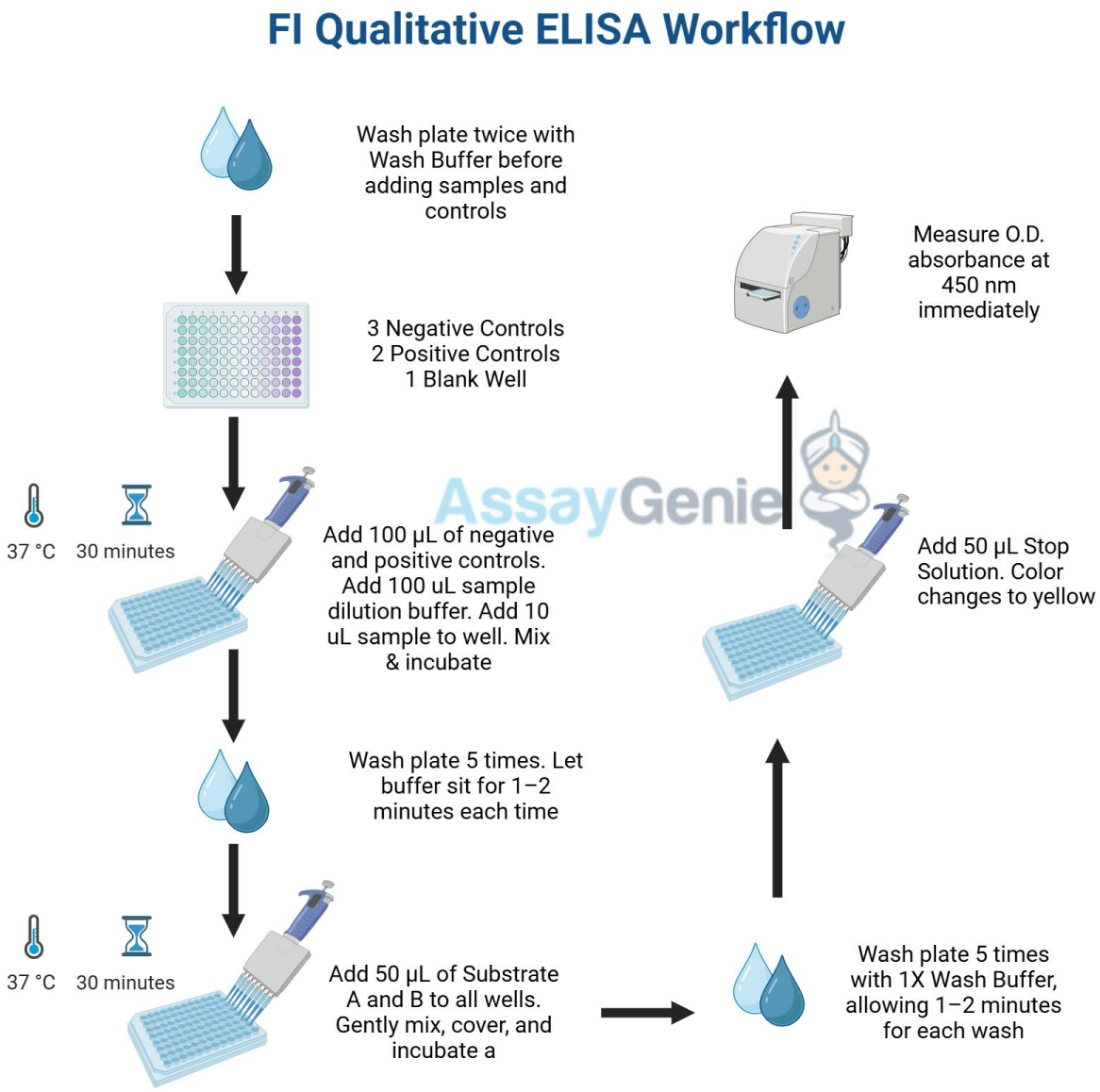
According to best practices, extract protein & perform the experiment as soon as possible after sample collection. Alternatively, store the extracts at the designated temperature (-20°C/-80°C). For optimal results, avoid repeated freeze-thaw cycles.

Serum: Coagulate the serum at room temperature (about 1 hours). Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20℃.

Notes

Samples stored at 2-8°C should be used within 5 days, samples stored at -20°C should be assayed within 1 month and samples stored at -80°C should be assayed within 2 months to reduce the loss of bioactivity. Avoid multiple freeze-thaw cycles. Hemolyzed samples are not suitable for this assay.

6. Workflow Overview



7. Reagent Preparation

Manual Washing

Discard the solution from the plate without touching the sides of the wells. Tap the plate firmly onto absorbent filter paper or another absorbent material. Completely fill each well with 350 µl of wash buffer and let it soak for 1 to 2 minutes. Then aspirate the contents from the plate and tap it again onto absorbent filter paper or other absorbent material. Repeat this process for the specified number of washes.

Automated Washing

Aspirate all wells, then wash the plate three times with 350 µl of wash buffer per well. After the final wash, invert the plate and tap it firmly on absorbent filter paper or another absorbent material. It is recommended to set the washer with a 1-minute soak time.

Reagent Preparation

Bring all reagents and samples to room temperature 20 minutes before use.

Wash Buffer:

Dilute 50mL of Concentrated Wash Buffer into 950 mL of Wash Buffer with deionized or distilled water. (Crystals that form in the concentrated wash buffer can be dissolved by placing the solution in a water bath at 40°C until fully dissolved (do not exceed 50°C). Mix well before proceeding to the next step. It is recommended to use the prepared wash buffer within one day. Any remaining buffer can be stored at 2-8°C for up to 48 hours).

8. Assay Procedure

1- Wash the plate twice before adding samples and controls.

2- Label the wells for samples, 3 Negative Controls, 2 Positive Controls, and 1 blank well.

3- Add 50 µL of Negative Control and Positive Control solutions to each respective well (except the blank well).

4- Remove the cover and wash the plate 5 times with 1X Wash Buffer, allowing the buffer to sit in the wells for 1–2 minutes each time.

5- Add 50 µL of HRP-conjugated antigen solution to each well containing controls and samples. Add the solution to the bottom of each well without touching the side walls.

6- Cover the plate and incubate at 37°C for 30 minutes.

7- Remove the cover and wash the plate 5 times with 1X Wash Buffer, allowing the buffer to sit in the wells for 1–2 minutes each time.

8- Add 50 µL of TMB Substrate A and 50 µL of TMB Substrate B to each well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37°C in the dark for 15 minutes. A blue color should develop in the Positive Control wells, while Negative Control wells should show no noticeable color.

9- Add 50 µL of Stop Solution to each well and mix thoroughly. The color should immediately change to yellow.

10- Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the Stop Solution (using the blank well to set the zero reference).

9. Data Analysis

1. **Cut-off Value** = NCx+0.1

NCx: Mean Absorbance of Negative Control (A)

*If the mean absorbance (A) value for the negative control is less than 0.05, it should be recorded as 0.05. If the negative mean A value is 0.05 or greater, record the actual value.

*Positive Control (PC): If PC≤ 0.3, the test is considered invalid and should be repeated.

*The absorbance of the blank well (containing only TMB and Stop Solution) should not exceed 0.08

2. Determination of results

Sample with absorbance values ≤ Cutoff Value are NON-REACTIVE and are considered NEGATIVE. Sample with absorbance values > Cutoff Value are considered POSITIVE.

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If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

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