Goat/Sheep TOX-IgG (Toxoplasma-Immunoglobulin G) ELISA Kit

Catalogue No.: EGS0001

Size: 96T

Reactivity:Goat/Sheep

Application: This immunoassay kit allows for the qualitative determination ofTOX-IgG inGoat/Sheep serum.

Storage: 2-8°C.

NOTE: FOR RESEARCH USE ONLY.

Kit Components

Item	Specifications(96T)	Storage
ELISA Microplate(Dismountable)	8×12	2-8°C
Negative Control (Ready-to-use)	0.5ml	2-8°C
Positive Control (Ready-to-use)	0.5ml	2-8°C
Sample Dilution Buffer	12ml	2-8°C
HRP-conjugated anti-Goat/Sheep IgG antibody	12ml	2-8°C
Wash buffer (20X)	50ml	2-8°C
TMB Substrate A	6ml	2-8°C (protect from light)
TMB Substrate B	6ml	2-8°C (protect from light)
Stop solution	6ml	2-8°C
Plate Sealer	3pieces	
Instruction manual	1copy	

Wuhan Fine Biotech Co., Ltd.

B9 Bld, High-Tech Medical Devices Park, No. 818 Gaoxin Ave. East Lake High-Tech Development Zone, Wuhan, Hubei, China(430206)

Tel: (0086)027-87384275 Fax: (0086)027-87800889 www.fn-test.com

Principle of the Assay

This kit was based on indirect enzyme-linked immune-sorbent assay technology.TOX-Ag was 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- Anti-Goat/Sheep IgG Antibody, if there were any TOX-IgG in the samples, it would form a TOX-Ag -TOX-IgG - HRP- Anti-Goat/Sheep IgG complex. 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.

Precautions for Use

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep plate dry.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive and high background.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don't let microplate dry at the assay, for dry plate will inactivate active components on plate.
8. Don't reuse tips and tubes to avoid cross contamination.
9. Please do not mix the reagents in different kits of our company. Do not mix reagents from other manuf acturers.
10. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

Material Required But Not Supplied

1.Microplate reader (wavelength: 450nm)

2.37°C incubator

3.Automated plate washer

4.Precision single and multi-channel pipette and disposable tips

Wuhan Fine Biotech Co., Ltd.

B9 Bld, High-Tech Medical Devices Park, No. 818 Gaoxin Ave. East Lake High-Tech Development Zone, Wuhan, Hubei, China(430206)

Tel: (0086)027-87384275 Fax: (0086)027-87800889 www.fn-test.com

5.Clean tubes and Eppendorf tubes

6.Deionized or distilled water

Manual Washing

Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350ul wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure two more times for a total of THREE washes.

Automated Washing

Aspirate all wells, then wash plate THREE times with 350ul wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 minute.

Sample Collection and Storage

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C for long term. Avoid multiple 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°C.

Note: Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this assay.

Wash Buffer Preparation:

Dilute 50mL of Concentrated Wash Buffer into 1000 mL of Wash Buffer with deionized or distilled water.

Assay Procedure

1. Bring all reagents to room temperature before use.

2. Label the sample wells, 3 Negative Controls, 1 Positive Controls and 1 blank well.

3. Add 100ul sample dilution buffer to each sample well.

4. Add 10ul sample to sample well.

Wuhan Fine Biotech Co., Ltd.

B9 Bld, High-Tech Medical Devices Park, No. 818 Gaoxin Ave. East Lake High-Tech Development Zone, Wuhan, Hubei, China(430206)

Tel: (0086)027-87384275 Fax: (0086)027-87800889 www.fn-test.com

5. Add 100ul Negative Control or Positive Control to Control wells and gently tap the plate to ensure thorough mixing.

6. Seal the plate with a cover and incubate at 37°C for 30 minutes.

7. Remove the cover, and wash plate 5 times with Wash buffer and each time let the wash buffer stay in the wells for 1 minute.

8. Add 100ul HRP-conjugated anti-Goat/Sheep IgG Working Solution to each well, except the blank well.

9. Seal the plate with a cover and incubate at 37°C for 30 minutes.

10. Remove the cover, and wash plate 5 times with Wash buffer and each time let the wash buffer stay in the wells for 1 minute.

11. Add 50ul of TMB substrate A and 50ul of TMB substrate B into each well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37°C in dark for 10 minutes. And the shades of obvious blue can be seen in the Positive Controls. Blank well wells show no obvious color.

10. Add 50ul of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.

11. Read the O.D. absorbance at 450nm in a microplate reader immediately after adding the stop solution (Use the blank well to set zero).

Data Analysis

NCx: Mean Absorbance of Negative Control (A)

Cutoff Value = NCx+0.10

Quality control

NCx: A ≤0.08 (If A ≤0.05, set NCx as 0.05. If 0.05＜A ≤0.08, set NCx to the actual value. If A>0.08, it should be retested)

Positive control: A ≥ 0.3

Calculation of Results

1. Sample with absorbance values < Cutoff Value are considered negative.

Sample with absorbance value ≥ Cutoff Value are considered positive.

2. PCx≤0.3, the testis regarded as invalid, should be tested again.

Wuhan Fine Biotech Co., Ltd.

B9 Bld, High-Tech Medical Devices Park, No. 818 Gaoxin Ave. East Lake High-Tech Development Zone, Wuhan, Hubei, China(430206)

Tel: (0086)027-87384275 Fax: (0086)027-87800889 www.fn-test.com