Gen (Gentamicin) ELISA Kit

Catalog No: E-FS-E073

96T/96T*3

Version Number:	V1.5
Replace version:	V1.4
Revision Date:	2026.11.02

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.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.elabscience.com

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

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Test principle

This kit uses Competitive-ELISA as the method for the quantitative detection. It can detect gentamicin (Gen) in samples, such as muscle, milk, egg, bovine and ovine liver etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody working solution, standard and other supplementary reagents. The microtiter plate provided in this kit has been pre-coated with coupled antigen. During the detection, Gen in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-Gen antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each well, and substrate reagent is added for color development. There is a negative correlation between the OD value of samples and the concentration of Gen. The concentration of Gen in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Reaction mode (Incubation time and temperature)**:** 25±2℃; 30 min, 15-20 min.

Detection limit: Muscle, Egg---10 ppb; Raw milk, Finished milk ---15 ppb,
bovine liver, ovine liver---50 ppb.

Cross-reactivity: Gentamicin ---100%;
Streptomycin, Neomycin, Kanamycin, Dihydrostreptomycin --- < 0.01%.

Sample recovery rate: 90%±30%.

Kits components

Item	Specifications
ELISA Microtiter plate	96/96*3 wells
Standard Liquid	1.5/1.5*3 mL each (ppb=ng/mL=ng/g) (0 ppb, 0.1 ppb, 0.2 ppb, 0.6 ppb, 1.8 ppb, 5.4 ppb)
HRP Conjugate	7/7*3 mL
Antibody Working Solution	7/7*3 mL
Substrate Reagent A	7/7*3 mL
Substrate Reagent B	7/7*3 mL
Stop Solution	7/7*3 mL
20×Concentrated Wash Buffer	25/25*3 mL
10×Concentrated Egg Diluent	7/7*3 mL
4×Concentrated Sample Diluent	25/25*3 mL
10×Concentrated Tissue Extraction Solution	100/100*3 mL
Plate Sealer	1/1*3 piece
Sealed Bag	1/1*3 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Othermaterials required but not supplied

Instruments: Microplate Reader, Homogenizer, Vortex mixer, Centrifuge, Balance (sensibility 0.01 g).
Micropipette: Single Channel (20-200 μL, 100-1000 μL), Multichannel (30-300 μL).
Reagents: Trichloroacetic Acid ($C_2HCl_3O_2$), NaCl.

Notes

1. The overall OD value will be lower when reagents have not been brought to room temperature before use or room temperature is below 25±2℃.
2. If the wells turn dry during the washing procedure, it will lead to bad linear standard curve and poor repeatability. Operate the next step immediately after wash.
3. Mix thoroughly and wash the plate completely. The consistency of wash procedure can strongly affect the reproducibility of this ELISA kit.
4. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid th e kit to strong light.
5. **Each reagent is optimized for use in the E-FS-E073. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E073 with different lot numbers**.
6. Substrate Reagent should be abandoned if it turns blue color. When OD value of standard (concentration: 0) < 0.8 unit (A450nm < 0.8), it indicates the reagent may be deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.
8. As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.
9. 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.
10. **For mentioned sample fast and efficient extraction methods are included in the kit descriptio n. Please consult technical support for the applicability if other sample need to be tested.**
11. The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS, etc. can be used for quantitative confirmation.

Storage and expiry date

Store the kit at 2-8℃. Do not freeze any test kit components.
Return any unused microwells to their original foil bag and reseal them together with the desiccant provided and further store at 2-8℃. After opening, the kit is stable for up to 1 month.
Expiry date: expiration date is on the packing box.

Experimental preparation

Restore all reagents and samples to room temperature before use.

Open the microplate reader in advance, preheat the instrument, and set the testing parameters.

1. **Sample pretreatment Notice:**

   Experimental apparatus should be clean, and the pipette should be disposable to avoid cross-contamination during the experiment.

2. **Solution preparation**

   Please prepare solution according to the number of samples. Don't use up all components in the kit at once!

   Solution 1: **1%Trichloroacetic Acid Solution** *(for raw milk, finished milk, bovine liver, ovine liver sample)*

   Dissolve 1 g of **Trichloroacetic Acid** to 100 mL of deionized water, mix fully.

   Solution 2: **4% Trichloroacetic Acid Solution** *(for egg sample)*

   Dissolve 8 g of **Trichloroacetic Acid** to 200 mL of deionized water, mix fully.

   Solution 3: **Sample Diluent** *(for raw milk, finished milk, bovine liver, ovine liver sample)*

   Dilute **4×Concentrated Sample Diluent** with deionized water. (4×Concentrated Sample Diluent (V): Deionized water (V) = 1:3).

   Solution 4: **Tissue Extraction Solution** *(for livestock sample)*

   Dilute 100 mL of **10×Concentrated Tissue Extraction Solution** with 900 mL of deionized water, add 20 g of **NaCl**, mix fully.

   Solution 5: **Egg Diluent**

   Dilute **10×Concentrated Egg Diluent** with deionized water. (10×Concentrated Wash Buffer (V): Deionized water (V) = 1:9).

   Solution 6: **Wash Buffer**

   Dilute **20×Concentrated Wash Buffer** with deionized water. (20×Concentrated Wash Buffer (V): Deionized water (V) = 1:19).

3. **Sample pretreatment procedure**

3.1 Pretreatment of muscle (pork, beef, mutton, chicken, duck) sample:

(1) Remove fat from sample. Homogenize the representative sample with a homogenizer and mix fully.

(2) Weigh 1±0.05 g of homogenate sample into 50 mL centrifuge tube.

(3) Add 10 mL **Tissue Extraction Solution** (Solution 4). Vortex for 1 min, centrifuge at 4000 g for 5 min at room temperature.

(4) Take 50 µL of the supernatant for analysis.

Note: Sample dilution factor: 10, detection limit: 10 ppb

3.2 Pretreatment of raw milk, finished milk sample:

(1) Weigh 1±0.05 g of sample in to 5 mL centrifuge tube, and add 1 mL of **1% Trichloroacetic Acid Solution** (Solution 1), vortex for 1 min, mix fully.

(2) Centrifuge at 4000 g for 5 min at room temperature.

(3) Take 50 µL of intermediate liquid into 2 mL centrifuge tube. Add 950 µL of **Sample Diluent** (Solution 3), vortex for 30 s, mix fully.

(4) Take 50 µL for analysis.

Note: Sample dilution factor: 40, detection limit: 15 ppb

3.3 Pretreatment of egg sample:

(1) Weigh 2±0.05 g of homogenized sample into 10 mL centrifuge tube, and add 3 mL of **4% Trichloroacetic Acid Solution** (Solution 2), vortex for 2 min, mix fully.

(2) Centrifuge at 4000 g for 5 min at room temperature.

(3) Take 100 µL of supernatant into 2 mL centrifuge tube. Add 500 µL of **Egg Diluent** (Solution 5), vortex for 10 s, mix fully.

(4) Take 50 µL for analysis.

Note: Sample dilution factor: 15, detection limit: 10 ppb

3.4 Pretreatment of bovine liver, ovine liver sample:

(1) Homogenize the representative sample with a homogenizer and mix fully.

(2) Weigh 1±0.05 g of homogenate sample into 50 mL centrifuge tube, and add 10 mL of **1% Trichloroacetic Acid Solution** (Solution 1), vortex for 1 min, mix fully.

(3) Centrifuge at 4000 g for 5 min at room temperature, Take 50 µL of intermediate liquid into 2 mL centrifuge tube. Add 950 µL of **Sample Diluent** (Solution 3), vortex for 30 s, mix fully.

(4) Take 50 µL of the supernatant for analysis.

Note: Sample dilution factor: 200, detection limit: 50 ppb

Assay procedure

Restore all reagents and samples to room temperature before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8℃.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells.**Standard and Samples need test in duplicate**.
2. **Add Sample:** add 50 μL of **Standard** or **Sample** per well, add 50 μL of **HRP Conjugate**, then add 50 μL of **Antibody Working Solution** into each well. Gently oscillate for 10 s to mix thoroughly and cover the plate with sealer. Incubate for 30 min at 25±2℃ in shading light.
3. **Wash:** uncover the sealer carefully, remove the liquid of each well. Immediately add 260 μL of **Wash Buffer** (Solution 6). Repeat wash procedure for 4 times, 15-30 s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **Color Development:** add 100 μL of **Substrate mixed solution** (**Substrate Reagent A** and **Substrate Reagent B** are mixed 1:1 by volume, must be fully mixed, the mixture should be used within 5 min, avoid the use of metal container, stirring reagents.) to each well, gently oscillate for 10 s to mix thoroughly. Incubate at 25±2℃ for 15-20 min in shading light.
5. **Stop Reaction:** add 50 μL of **Stop Solution** to each well. Gently oscillate for 10 s to mix thoroughly
6. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm (reference wavelength 630 nm) with a microplate reader. This step should be finished in 5 min after stop reaction.

Result analysis

1. **Absorbance% =A/A_0×100%**

   A: Average absorbance of standard solution or sample

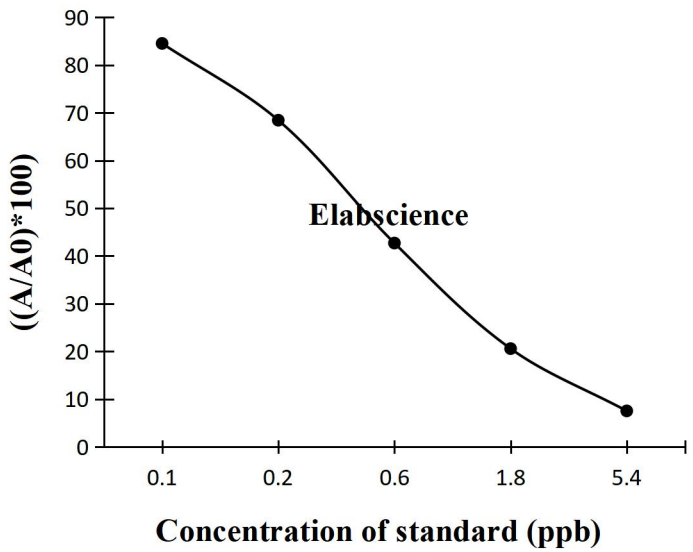
   A_0: Average absorbance of 0 ppb Standard solution

2. **Drawing and calculation of standard curve**

   Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add the average absorbance value to standard curve to get corresponding concentration. **If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.**

   For this kit, it is more convenient to use professional analysis form for accurate and fast analysis on a large number of samples.

Gentamicin (E-FS-E073) **Standard Curve**



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