RAC (Ractopamine) ELISA Kit

Catalog No: E-FS-E012

96T/96T*3

Version Number: V1.2

Replace version: V1.1

Revision Date: 2026.04.20

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.

Copyright ©2023-2024 Elabscience Bionovation Inc. All Rights Reserved

Test principle

This kit uses Competitive-ELISA as the method for the quantitative detection. It can detect Ractopamine (RAC) in samples, such as muscle, feed, Liver, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody working solution, standard and other supplementary reagents. The microtiter plate in this kit has been pre-coated with coupled antigen. During the reaction, RAC in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-RAC antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microtiter plate well, and substrate reagent is added for color development. There is a negative correlation between the OD value of samples and the concentration of RAC. The concentration of RAC 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°C; 30 min, 10 min.

Detection limit: Porcine urine, pork, beef, mutton---approx. 0.5 ppb; pork liver, bovine urine, ovine urine---approx. 1 ppb; feed---approx. 5 ppb

Cross-reactivity: Ractopamine---100%; Dobutamine---4.7%; Ritodrine---14%; Mabuterol---4.4%; Clenproperol, Cimaterol---<0.1%; Cimbuterol, Mapenterol, Terbutaline, Salbutamol, Clenpenterol, Clenbuterol, Brombuterol, Cabuterol, Bromoclenbuterol ---<0.01%.

Sample recovery rate: 90% ± 30%.

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1.5 mL each (ppb=ng/mL=ng/g) (0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb)
HRP Conjugate	7 mL
Antibody Working Solution	7 mL
20×Concentrated Sample Diluent	4 mL
20×Concentrated Wash Buffer	25 mL
Substrate Reagent A	7 mL
Substrate Reagent B	7 mL
Stop Solution	7 mL
Plate Sealer	1 piece
Sealed Bag	1 piece
Manual	1 copy

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

Other materials required but not supplied

Instruments: Microplate reader, Printer, Homogenizer, Nitrogen, Water bath, Vortex mixer, Centrifuge, Graduated pipette, Balance (sensibility 0.01 g).

Micropipette: Single channel (20-200 μL, 100-1000 μL), Multichannel (30-300 μL).

Reagents: NaOH, Conc. HCl, Trichloroacetic acid.

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. 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 the kit to strong light.
5. **Each reagent is optimized for use in the E-FS-E012. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E012 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue color. When OD value of standard (concentration: 0) < 0.5 unit (A450nm < 0.5), it indicates the reagent 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 micro-plate 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: **1M NaOH**

   Weigh 4 g of NaOH, dissolve in deionized water, and dilute to 100 mL.

   Solution 2: **0.5M NaOH**

   Weigh 2 g of NaOH, dissolve in deionized water, and dilute to 100 mL.

   Solution 3: **1M HCl**

   Measure 8.6 mL of Conc. HCl, add deionized water to make the volume up to 100 mL.

   Solution 4: **Tissue Diluent**

   Weigh 1 g of Trichloroacetic acid (TCA), add 100 mL of deionized water and dissolve thoroughly.

   Solution 5: **Wash Buffer**

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

   Solution 6: **Sample Diluent**

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

3. **Sample pretreatment procedure**

3.1 Pretreatment of porcine urine sample:

(1) If turbid, filter or centrifuge at 4000 g for 5 min.

(2) Take 30 μL of liquid for analysis.

Note: Sample dilution factor: 1, detection limit: 0.5 ppb.

3.2 Pretreatment of bovine urine, ovine urine sample:

(1) If turbid, filter or centrifuge at 4000 g for 5 min.

(2) Take 500 μL of bovine/ovine urine, add 500 μL of **Wash Buffer** (Solution 5), and vortex thoroughly for 1 min.

(3) Take 30 μL for analysis.

Note: Sample dilution factor: 2, detection limit: 1 ppb.

3.3 Pretreatment of tissue (pork, beef, mutton, pork liver) sample:

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

(2) Weigh 2±0.05 g of crushed homogenate, add 3 mL of **Wash Buffer** (Solution 5), add 3 mL of **Tissue Diluent** (Solution 4). Vortex fully for 1 min, centrifuge at 4000 g for 5 min.

(3) Transfer 1 mL of the intermediate clear supernatant into a new centrifuge tube (Avoid aspirating solids from the upper and lower layers, as this may affect the test results), add 50 μL of **0.5M NaOH** (Solution 2). Vortex fully for 10 seconds, centrifuge at 4000 g for 5 min.

(4) Take 30 μL of the supernatant for analysis.

Note: Sample dilution factor: 4, detection limit: pork, beef, mutton--0.5 ppb; pork liver--1 ppb.

3.4 Pretreatment of feed sample:

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

(2) Weigh 1±0.05 g of homogenate feed sample to 50 mL centrifuge tube, add 1 mL of **1M HCl** (Solution 3), add 9 mL of deionized water, vortex for 1 min, shake on a shaker at 300 rpm for 15 min. Centrifuge at 4000 r/min for 10 min at room temperature.

(3) Take 0.1 mL of the supernatant to another centrifuge tube. Add 65 μL of **1M NaOH** (Solution 1), vortex for 30 seconds to mix fully. Centrifuge at 4000 r/min for 10 min at room temperature.

(4) Take 200 μL of the supernatant into a new centrifuge tube, add 400 μL of **Sample Diluent** (Solution 6), and vortex thoroughly for 30 seconds.

(5) Take 30 μL for analysis.

Note: Sample dilution factor: 32, detection limit: 5 ppb.

Assay procedure

Restore all reagents and samples to room temperature (25℃) 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 30 μL of **Standard or Sample** per well, then add 50 μL **HRP Conjugate** to each well. Add 50 μL **Antibody Working Solution**, cover the plate with plate sealer, and oscillate for 10 seconds gently to mix thoroughly. Incubate at 25±2℃ for 30 min in shading light.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 260 μL of **Wash Buffer** (Solution 5) to each well and wash. Repeat wash procedure for 4 times, 30 seconds 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 to each well (**Substrate Reagent A** and **Substrate Reagent B** are fully mixed at ratio 1:1 by volume, the mixture should be used within 5 min, avoid using metal containers or stirring the reagents), Gently oscillate for 10 seconds to mix. thoroughly. Incubate at 25±2℃ for 10-15 min in shading light.
5. **Stop reaction:** add 50 μL of **Stop Solution** to each well, gently oscillate and mix fully to stop the reaction.
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

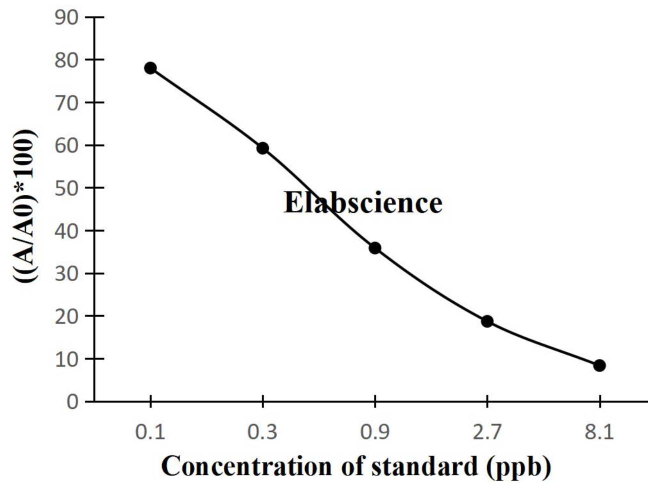
   A_0: Average absorbance of 0 ppb Standard solution

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 of sample 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 many samples.

Ractopamine (E-FS-E012) **Standard Curve**



Copyright ©2023-2024 Elabscience Bionovation Inc. All Rights Reserve