

Ractopamine ELISA Kit

05/18

(Catalog # E4565-100, 100 assays, Store at 4°C)

I. Introduction:

Ractopamine (RA) is a synthetic beta-adrenoreceptor agonist. It is used in the treatment of congestive heart failure and muscular dystrophy, increase muscle growth, decrease fat deposition and is beneficial for the growth of fetus and newborn. Ractopamine is used as a new type of Clenbuterol by some pig farms, but it has been banned in most countries. This kit is a detection product developed based on ELISA technology, with operation time as short as 50 min and a sensitivity of 0.1 ppb, and linear range from 0.1 ppb to 8.1 ppb.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Ractopamine.

Detection Range: 0.05 – 4.05 ppb (ng/ml)

Sensitivity: 0.05 ppb (ng/ml)

Detection limitation: 0.5 ppb for urine, 0.25 ppb for pork, beef and liver, 5 ppb for feed

Cross Reactivity: Clenbuterol < 0.1%, Salbutamol < 0.1%

III. Sample Type:

Urine, pork, liver, feed

IV. Kit Contents:

Components	E4565-100	Part No.
Micro ELISA Plate	8 X 12 Strips	E4565-100-1
Standard (S0 – S5)	1 ml X 6	E4565-100-2
HRP-conjugate	7 ml	E4565-100-3
Antibody	7 ml	E4565-100-4
TMB substrate	12 ml	E4565-100-5
Stop Solution	10 ml	E4565-100-6
Sample Diluent	50 ml	E4565-100-7
Extraction Solution 1	500 ml	E4565-100-8
Extraction Solution 2 (20X)	15 ml X 2	E4565-100-9
1M HCl	15 ml	E4565-100-10
Wash Buffer (10X)	30 ml	E4565-100-11
Plate sealers	4	E4565-100-12

V. User Supplied Reagents and Equipment:

- Methanol, 1M HCl
- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment. Opened kit may be stable for 1 month at 4°C.

VII. Reagent and Sample Preparation:

Note: Bring all reagents to room temperature (20-25°C) 30 minutes before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes. Finish preparing reagent 10 minutes before the assay.

1. **Extraction Solution 2** (for liver sample): Add 25 ml of ExtractionSolution (20×) to 475 ml of deionized water, shake well.
2. **Extraction Solution 3** (for feed sample): Add 10 ml of 1M HCl to 990 ml deionized water, shake well
3. **Wash Buffer (1X):** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals are completely dissolved. Dilute 10 ml of Wash Buffer (10X) into 90 ml deionized water to prepare 100 ml of Wash Buffer (1X). Can be stored at 4°C for one month.
4. **Standards Concentration:** Ready to use

Standards	S0	S1	S2	S3	S4	S5
Concentration (ppb)	0	0.05	0.15	0.45	1.35	4.05

5. Sample Preparation:

Note: The prepared sample maybe stored for up to one day at 2-8°C.

- Urine

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1. Balance to room temperature and take 50 µl urine for test. If the urine is not clear, centrifuge at 4000 rpm for 10 min and take supernatant for future analysis. (Dilution factor: 1)

- **Tissue (pork, beef)**

1. Weigh 1.0 g of the homogenized sample and put into centrifugal tube.
2. Add 5 ml of Extraction Solution 1 and vortex properly for 1 min. Centrifuge at 4000 rpm for 10 min.
3. Take 50 µl of sample for future analysis. (Dilution factor 5)

- **Tissue (liver)**

1. Weigh 1.0 g of the homogenized sample and put into centrifugal tube, add 5 ml of Extraction Solution 2 and vortex properly for 1 min and centrifuge at 4000 rpm for 10 min.
2. Transfer 1 ml of supernatant and add 20 µl of 1M HCl, shake properly for 1 min.
3. Centrifuge at 4000 rpm for 10 min.
4. Take 50 µl of supernatant sample for future analysis. (Dilution factor 5)

- **Feed**

1. Weigh 1.0 g of the homogenized sample, put into centrifugal tube.
2. Add 5 ml of Extraction Solution 3 and 5 ml of Methanol. Vortex properly for 10 min.
3. Centrifuge at 4000 rpm for 10 min
4. Transfer 50 µl supernatant into a new centrifugal tube, add 200 µl of Sample Diluent, shake well.
5. Take 50 µl sample for further analysis.
6. Dilution factor of the samples: 50

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VII. Store unused wells back to 2-8°C.
2. Add 50 µl of **Standard** or **Sample** per well. Then add 50 µl of **HRP-conjugate** to each well and 50 µl of **Antibody** to each well. Cover the microtiter plate with a new adhesive strip and mix well, then incubate for 30 min at 25°C.
3. Aspirate each well and wash, repeating the process 4 times. Wash by filling each well with 250 µl of **Wash Buffer** using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 30 seconds, complete removal of liquid at each step is essential to good performance.
4. Add 100 µl of **TMB Substrate** to each well, mix well. Incubate for 15 minutes at 25°C. Protect from light.
5. Add 50 µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
6. Read result at 450 nm within 5 minutes.

IX. CALCULATION:

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

$$\text{Absorbance Value (\%)} = \frac{B}{B_0} \times 100\%$$

B: The average absorbance value of the sample or standard; B₀: The average absorbance value of the 0 ppb standard

To draw a standard curve: Take the absorbency value of standards as y-axis, logarithmic of the concentration of the Ractopamine standards solution (ppb) as x-axis. The Ractopamine concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

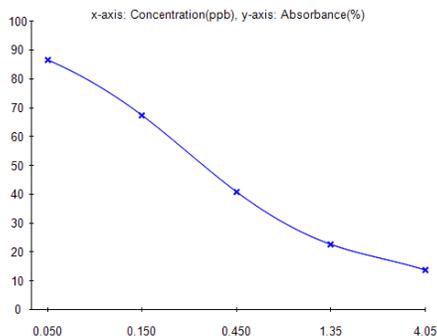


Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay

X. RELATED PRODUCTS:

- Clenbuterol ELISA Kit ((Cat. No. E4564)
- Salbutamol (SALB) ELISA Kit (Cat. No. K4209)
- Chloramphenicol (CAP) ELISA Kit (Cat. No. K4230)
- Ciprofloxacin (Cipro) ELISA Kit (Cat. No. E4365)

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- Enrofloxacin (ENR) ELISA Kit (Cat. No. E4277)
- Fluoroquinolones ELISA Kit (Cat. No. E4275)

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