Importance of the Ractopamine Determination

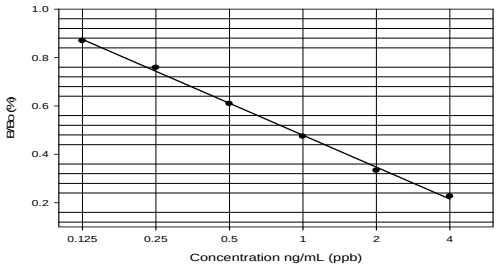
Ractopamine is a phenolethanolamine β-adrenoreceptor agonist that is approved in the US for use in pigs to improve weight gain, carcass leanness and feed efficiency in pigs. Ractopamine has a history of off-label use by livestock producers hoping to improve the economics of livestock production. Ractopamine use is forbidden in EU and some Asian countries.

The presence of drug residues in animal tissues is a concern for food safety, especially when the compound has been used illegally or in a manner not approved by regulatory officials. In an effort to combat the illicit use of Ractopamine, regulatory organizations world-wide test animal tissues or excreta for the presence of its illicit use. For regulatory purposes, both screening and confirmatory assays are used to detect illegal residues.

The Ractopamine ELISA allows the determination of 41 samples in duplicate determination. Only a few milliliters of sample are required. The test can be performed in less than 2 hours.

Performance Data

Test sensitivity: The detection limit for Ractopamine is 0.10 ng/mL (mean of 6 blank determinations minus 3 SD). The middle of the test (50% B/B_0) is at approximately 0.80 ng/mL. Determinations closer to the middle of the tests gives the most accurate results.



Test reproducibility: Coefficients of variation (CVs) for standards: <10%, CVs for samples: <15%.

Selectivity: This ELISA recognizes Ractopamine and other related compounds with varying degrees:

Cross-reactivities:		
	Ractopamine	100% (per definition)
	Salmeterol	0.02%
	Terbutaline	<0.012%
	Clenbuterol	<0.012%
	Sabutamol	<0.012%
	Epinephrine	<0.012%

Cross-reactivities with non-related compounds have not been observed.

Samples: Swine and bovine meat (muscle and liver) samples.

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For ordering or technical assistance contact:

Abraxis LLC
54 Steamwhistle Drive
Warminster, PA 18974
Tel.: (215) 357-3911
Fax: (215) 357-5232
Email: info@abraxiskits.com
WEB: *WWW.abraxiskits.com*

R051508

Ractopamine ELISA, Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of Ractopamine in Meat and other Contaminated Samples

Product No. 515555

1. General Description

The Ractopamine ELISA is an immunoassay for the quantitative and sensitive detection of Ractopamine in meat. Other sample matrices can also be tested using this ELISA but they should be validated. Ractopamine is a member of the family of β-adregenic agonists (β-agonists). This class of compounds has become notable for their ability to enhance the growth rate of farm animals by promoting the repartitioning of fat into muscle. In the case of Ractopamine, these effects have been most noticeable in pigs, but other species such as cattle and fish have also shown similar effects. There are many documented cases where the illegal use of this family of compounds has resulted in human food poisoning. The MRL of Ractopamine varies by country. The E.U. has a zero tolerance level. The U.S. MRL for products from swine is 1.2 ppm in liver and 0.05 ppm in muscle and other contaminated samples. If necessary, positive samples can be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

The standard solutions in the test kit contain small amounts of Ractopamine. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of stopping solution with skin and mucous membranes. If these reagents come in contact with the skin, wash with water.

3. Storage and Stability

The Ractopamine ELISA should to be stored in the refrigerator (4–8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box.

4. Test Principle

The test is a direct competitive ELISA based on the recognition of Ractopamine by specific antibodies. Ractopamine, when present in a sample and a Ractopamine-enzyme-conjugate compete for the binding sites of rabbit anti-Ractopamine antibodies in solution. The Ractopamine antibodies are then bound by a second antibody (goat anti-rabbit) immobilized on the plate. After a washing step and addition of the substrate solution, a color signal is produced. The intensity of the blue color is inversely proportional to the concentration of the Ractopamine present in the sample. The color reaction is stopped after a specified time, and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the Ractopamine ELISA, Possible Test Interference

Mistakes in handling the test can cause errors. Possible sources for such errors can be:

Inadequate storage conditions of the test kit, wrong pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

Swine and cattle muscle samples have been tested using the extraction procedure listed in the sample preparation section and found to give recoveries between 85-120%.

The Abraxis Ractopamine ELISA kit provides screening results. As with any analytical technique (GC, HPLC, etc.) positive samples requiring some action should be confirmed by an alternative method.

Working Instructions

A. Materials Provided

1. Microtiter plate coated with a second antibody (goat anti-rabbit)
2. Standards (7): 0, 0.125, 0.25, 0.50, 1.0, 2.0, 4.0 ng/mL (ppb)
3. Antibody Solution (rabbit anti-Ractopamine), 6 mL
4. Ractopamine-HRP Conjugate, 6 mL
5. Sample Diluent Concentrate (10X), 25 mL. Use to dilute samples
6. Wash Solution (5X) Concentrate, 100 mL
7. Color Solution (TMB), 16 mL
8. Stop Solution, 2 X 6 mL

B. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. We recommend using a multi-channel pipette or a stepping pipette for adding the antibody, the conjugate, the substrate solution, and the stop solution in order to equalize the incubations periods of the standard solutions and the samples on the entire microtiter plate. Please use only the reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the foil bag. The remaining strips must be stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (4-8°C).
3. The standard solutions, enzyme conjugate, antibody, substrate, and stop solution are ready to use and do not require any further dilutions.
4. Dilute the Wash Buffer at a ratio of 1:5. If using the entire bottle (100 mL) add to 400 mL of deionized or distilled water. Dilute the Sample Diluent at a ratio of 1:10 with deionized or distilled water.
5. The stop solution must be handled with care, as it contains diluted H_2SO_4.

C. Assay Procedure

1. Add 50 µL of the standard solutions or the samples (extracts) into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
2. Add 50 µL of enzyme conjugate solution to the individual wells successively using a multi-channel pipette or a stepping pipette.
3. Add 50 µL of antibody solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop for about 30 seconds. Be careful not to spill contents.
4. Incubate the strips for 60 min at room temperature.
5. Wash the strips four times using the washing buffer solution. Please use at least a volume of 250 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
6. Add 150 µL of substrate solution to the wells. The strips are incubated for 45 min at room temperature. Protect the strips from direct sunlight.
7. Add 100 µL of stop solution to the wells in the same sequence as for the substrate solution.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

D. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-Parameter (preferred) or Logit/Log). For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the $\%B/B_0$ for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the $\%B/B_0$ for each standard on a vertical linear (y) axis versus the corresponding Ractopamine concentration on horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for samples will then yield levels in ppb of Ractopamine by interpolation using the standard curve.

Samples showing lower concentrations of Ractopamine compared to standard 1 (0.125 ng/mL) are considered to be negative. Samples showing a higher concentration than standard 6 (4.0 ng/mL) must be diluted further to obtain accurate results.

E. Additional Materials (not delivered with the test kit)

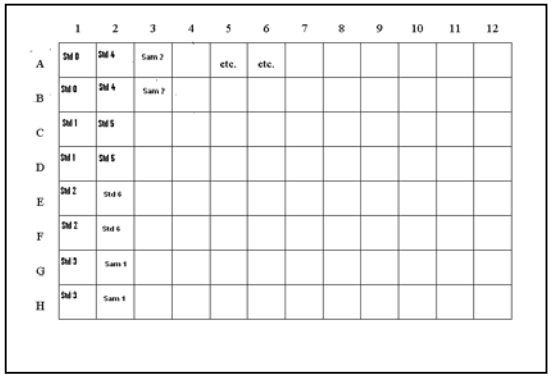
1. Micro-pipettes with disposable plastic tips (10-200, and 200-1000 µL)
2. Multi-channel pipette (10-250 µL) or stepper pipette with plastic tips (10-250 µL)
3. Microtiter plate washer
4. Microtiter plate reader (wave length 450 nm)
5. Shaker for microtiter plates (optional)

F. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

Std 0-Std 6: Standards
0; 0.125; 0.25; 0.50; 1.0; 2.0; 4.0 ppb

Sam1, Sam2, etc.: Samples



G. Preparation of Muscle and Liver Tissue Sample (Swine, Cattle)

1. 10 mL of 1X PBS is added to 10 g of muscle sample and homogenized for 10 minutes using a blender.
2. Pipette 1 mL of the homogenized sample into a plastic tube with cap, and add 3 mL of 3% TCA.
3. Mix using an overhead tube rotator for 30 minutes.
4. Centrifuge for 10 minutes at 2500 +/- 200 g.
5. Carefully remove 500 uL of the supernatant (upper layer) into a glass tube and add 200 uL of 1M phosphate buffer solution.
6. Dilute 1:10 in Sample Diluent (1X), i.e. 100 uL of supernatant (step 5) and 900 uL of Sample Diluent.
7. Analyze in the assay as sample (Assay Procedure step 1)
8. For highly contaminated samples (outside the range of the curve), we recommend further dilutions of 1:10 or 1:100 with sample dilution buffer (1X PBS).

8.1 The Ractopamine concentration in the samples is determined by multiplying the concentration of the extracts by a factor of 56 (this is the dilution factor introduced by the extraction/dilution procedure). If further dilutions are performed on the samples, this dilution factor needs to be multiplied by the sample value to obtain the final Ractopamine concentration on the sample.
NOTE: If lower detection limit are needed a modification of the procedure is performed, please contact Abraxis Technical Support.

H. Preparation of Solutions

1. 3%Trichloro acetic acid (TCA): 1.5 g of TCA (Sigma cat # T-6399) to 50 mL of distilled water, mix to dissolve.
2. 1M Phosphate Buffer: 14.2 g of sodium phosphate dibasic anhydrous to 100 mL of distilled or deionized water, mix to dissolve.
3. 1X PBS, pH 7.4: Sodium phosphate dibasic anhydrous 1.15 g; Sodium phosphate dibasic monohydrate 0.20 g; Sodium chloride 8.0 g; Potassium chloride 0.20 g to 1 liter of distilled or deionized water, pH 7.2-7.4.