Read instructions carefully before starting test

Alert® for Ractopamine

Refrigerate at 2–8°C (35–46°F)
Store controls at -20°C when not in use

USDA AMS Approved Verification Method
Alert® for Ractopamine has been evaluated by the United States Department of Agriculture, Agricultural Marketing Service and is in conformance with testing requirements for beta-agonists in meat and meat products.

RACTOPAMINE

Ractopamine is a beta-agonist used for increasing lean tissue development in livestock. Some regions and countries have banned ractopamine for use in livestock. Codex has defined a maximum residue limit (MRL) of ractopamine in pork and beef muscle at 10 parts per billion (ppb). Countries may have MRL levels that are more or less stringent than Codex.

INTENDED USE

Alert for Ractopamine is meant for the qualitative determination of trace quantities of ractopamine in muscle.

Neogen's enzyme-linked immunosorbent assay (ELISA) products are for screening purposes only. It is recommended that all suspect samples are confirmed by a quantitative method, such as mass spectrometry.

ASSAY PRINCIPLES

Alert for Ractopamine is a solid phase immunoassay. The test is performed in microwells coated with a specific antibody to the analyte. A control or sample is added to the wells followed by a ractopamine enzyme conjugate. During the following incubation period, the enzyme conjugate competes with the analyte in the sample for binding sites on the antibody coated well. After a wash step to remove any unbound material, substrate is added for the color development process. Stop solution is added to discontinue the enzyme-substrate reaction and to convert the product to the form, which is measured photometrically. Color intensity is inversely proportional to the amount of analyte present in the sample. In summary, those samples that contain the analyte will inhibit binding of the enzyme conjugate to the capture antibody, resulting in less intense color than the negative control.

Controls should be run along with the samples. Results should be obtained by reading the absorbance of the wells with a microplate reader.

INTENDED USER

Alert for Ractopamine is designed for use by personnel with appropriate training in general laboratory practices. Training in the use of this assay is available through Neogen.

MATERIALS PROVIDED

Kit Contents	Amount	Storage
Antibody-coated strips in well holder	1 x 96-well plate (8 wells x 12 strips)	2–8ºC
Ractopamine Standards: 0 ng/mL Muscle Standard 0.2 ng/mL Muscle Standard (Additional controls optional)	 1 mL 1 mL	-20ºC
Ractopamine Enzyme	1 vial, lyophilized	2–8ºC
Enzyme Diluent	16 mL	2–8ºC
Sample Diluent	125 mL	2–8ºC
10X Wash Solution	60 mL	2–8ºC
K-Blue® Substrate	13 mL	2–8ºC
Stop Solution	13 mL	2–8ºC

ADDITIONAL ITEMS REQUIRED BUT NOT PROVIDED

REAGENTS

1. Ethyl acetate, HPLC grade (Fisher E195-4)
2. Sodium carbonate, anhydrous (Fisher S263-1)
3. Distilled water
4. Sodium acetate, Bioxtra ≥ 99% (Sigma Cat# S-7545)
5. Acetic acid, ACS reagent ≥ 99.7% (Sigma-Aldrich# 695092)
6. β-glucuronidase Type HP-2 from Helix pomatia (Sigma Cat# G-7017)

MATERIALS

1. Disposable culture tubes or comparable vessels (glass preferred)
2. 5 mL or 10 mL serological pipettes (Neogen item 9415)
3. Pipettors and tips capable of delivering 50 µL and 100 µL (Neogen item 9276, 9407, 9417)
4. Well holder (Neogen item 9402) (**NOTE**: One well holder is included in kit, additional holders are necessary to run test)
5. Reagent troughs or weigh boats for dispensing reagents (Neogen item 9450 or 9435)
6. Pipettor 10–100 µL, 8-channel (**NOTE**: Not required if using automated liquid handling equipment) (Neogen item 9388, 9407, 9417)
7. Vortex with adapter set (Neogen item 9454)
8. Plate washer, multichannel pipettor or squirt bottle (Neogen item 9400, 9366)
9. Microplate reader, 450 nm wavelength absorbance filter (650 nm, 630 nm, or 620 nm wavelength reference filter) (Neogen item 9303)
10. 50 mL Polypropylene Centrifuge Tubes (Fisher 07-01-332)
11. 16 x 100 mm borosilicate disposable glass culture tubes and caps (Fisher 14-961-29 and 14-376-84)
12. Centrifuge, 4x50 mL Swing-Bucket Rotor; 110VAC (Cole-Palmer UX-17414-21)
13. Electric pipette aid (Fisher item 13-681-15)
14. Pipette pumps, 2–10 mL (Neogen item 9277)
15. Pipette pump, 2 mL (Fisher item 13-683-1B)
16. Thermometer (Neogen item 9461)
17. Timers, multichannel (Neogen item 9426)
18. Tube rack for 16 x 100 mm culture tubes, 20 positions (Fisher item 14-792-12)
19. Tube rack for 50 mL centrifuge tubes, 16 positions (Fisher item 14-791-6B)
20. Chemi-Scraper spatula (Fisher item 14-373)
21. Evaporator (Fisher item TS-18825, TS-18817, TS-188822)

PROCEDURAL NOTES (Recommendations)

1. **Ractopamine Enzyme**. Remove seal and stopper of the lyophilized ractopamine enzyme bottle, and reconstitute with exactly 14 mL enzyme diluent. It is recommended that a graduated pipette be used for greater accuracy. Replace the stopper, then swirl gently to mix. For optimum consistency and performance, an overnight (8 hours or longer) reconstitution time is recommended. However, **a minimum reconstitution time of 4 hours is required**. Prior to use, mix well by gentle inversion. Do not vortex. To prevent possible contamination of the reconstituted enzyme solution, aliquot the amount for immediate usage into a separate glass culture tube (transfer tube). From the transfer tube, pour into reagent trough and use multi-channel pipettor to dispense into wells.
2. **Sample diluent**. Ready-to-use, 125 mL bovine serum albumin in phosphate buffered saline (PBS).
3. **Wash solution, 10x**. Tween 20 in PBS. Dilute 1:10 with deionized water prior to use.

STORAGE

Store kits between 2–8°C and controls at -20°C when not in use.

The code number on the kit box and each kit component will have a different code designation that is a combination of the production date and kit item abbreviation.

The kit has a shelf life of 6 months from the date of production. Specific expiration dates for each kit are indicated on the kit box and Certificate of Analysis. The Ractopamine Enzyme's shelf life is one month after reconstitution.

PRECAUTIONS

1. Unless otherwise noted, refrigerate contents of the kit at 2–8˚C when not in use. Do not freeze reagents unless specifically instructed to do so.
2. Store controls at -20°C when not in use.
3. Prior to performing the test, allow for ELISA components, controls, and samples to reach room temperature (20–23°C). This usually takes about **60 minutes**. Reagents should not exceed 35°C.
4. If a precipitate is noted in a 10x solution after having been refrigerated, the precipitate should redissolve once the solution returns to room temperature. If not, dilute the entire amount of liquid and precipitate prior to use. Once diluted, the precipitate will redissolve.
5. Use new disposable pipette tips for each sample and control to avoid cross-contamination.
6. Do not mix reagents from a kit with one serial number with reagents from a kit with a different serial number.
7. The kit reagents contain anti-microbial agents as preservatives. Avoid contact with skin. If contact is made with skin, flush immediately with water.
8. Avoid contacting skin or eyes with stop solution. In case of contact, wash areas with large amounts of water.
9. The plate wells are for single use only. Each well can accommodate only one sample or control. Do not reuse wells.
10. Unused microwell strips must be stored in their original bag containing desiccant. Ensure that the bag is properly sealed.
11. Do not use sodium azide as a preservative in samples as it may adversely affect the enzyme.
12. Treat all samples and reagents as potentially infectious or biohazardous.

RECOMMENDATIONS

1. Neogen recommends using multiple wells for each control and sample.
2. Do not break strips.
3. Dispense only the amounts of reconstituted Ractopamine Enzyme and K-Blue Substrate needed for immediate testing into clean vessels. From these vessels, pipette to the wells of the plate. **Do not return excess reagents back into original bottles**. Contamination of these reagents may cause enzyme degradation or premature development of blue color in the substrate.
4. A good wash step is critical to achieving good well-to-well reproducibility. All liquids must be removed from the wells before applying substrate. After washing, the plate should be tamped on absorbent paper to remove any remaining residue.
5. Timing and speed of reagent addition can influence performance of the test. This is especially true for the addition of the K-Blue Substrate and stop solution. Substrate and stop solution should be applied to the plate wells as quickly as possible, preferably using a multichannel pipettor.
6. Complete all steps without interruption. Do not allow time to lapse between steps.

PROCEDURE SUMMARY

This procedure describes a method for the rapid qualitative screening of ractopamine in muscle. Homogenized tissue samples are extracted with low toxicity solvents using inexpensive processing equipment. Samples are tested using the ractopamine ELISA kit with a cutoff control. (Higher control concentrations optional.) Results are used as a guide for the identification of muscle tissues containing ractopamine.

Muscle tissue samples are initially ground and weighed into disposable containers. The sample's pH is adjusted with an acidic aqueous solution. The sample undergoes enzymatic hydrolysis through the addition of β-glucuronidase and is extracted with ethyl acetate. Samples are centrifuged, and a portion of the ethyl acetate phase is collected and reduced to dryness using heat and forced air. Dried samples are reconstituted in a sample dilution buffer and used directly for the test.

The test kit is based on an immunological reaction. The particular format of the test kit is a direct competitive ELISA. The absorbance responses of the extracted samples are compared to those of the supplied control. The response of the cutoff control is equivalent to 0.20 ppb ractopamine in muscle. A sample with an absorbance that is equal to or less than the supplied cutoff control is considered "positive" or containing greater than 0.20 ppb of ractopamine in muscle. A sample with an absorbance greater than the supplied cutoff control is likewise considered "negative" or containing less than 0.20 ppb of ractopamine in muscle. Results of the assay are to be considered as presumptive only.

SAMPLE PREPARATION

Buffers

A. Preparation of Sodium Acetate Buffer, pH 5.0

1. Dissolve 16.406 g of sodium acetate into 1800 mL of deionized water.
2. Adjust buffer's pH to 5.0 using glacial acetic acid.
3. Fill to 2 L using deionized water and store at 4°C.

B. Preparation of 25% Sodium Carbonate Buffer

1. Dissolve 25 g of sodium carbonate in 75 mL of deionized water.
2. Dilute to a final volume of 100 mL and store at room temperature.

Muscle

1. Remove excess fat and connective tissues from the muscle. Residual blood must also be removed by draining.
2. Cut the muscle into 3–4 inch pieces.
3. Grind the muscle chunks using a grinder or food processor to a puree consistency.

SAMPLE EXTRACTION

NOTE: This procedure should be performed in a hood or in a well-ventilated room.

1. Weigh 5 ± 0.1 g of muscle and add 5 ± 0.1 mL of sodium acetate buffer (pH 5.0) into 50 mL polypropylene centrifuge tube.
2. Add 8000 U β-glucuronidase and vortex vigorously for **30 seconds**.
3. Incubate for 2 hours at 60°C in a water bath.
4. Remove sample from water bath and add 5 ± 0.1 mL of 25% sodium carbonate solution.
5. Vortex the sample for a minimum of **60 seconds** at maximum speed.
6. Add 15 mL ethyl acetate.
7. Vortex the sample for a minimum of **60 seconds** at maximum speed.
8. Let the sample stand for **15 minutes** with occasional vortexing.
9. Centrifuge at 3000 rpm for **10 minutes**. A firm sample pellet must form.
10. Transfer 7.5 mL of upper ethyl acetate layer into a 16x100 mm disposable glass tube.
11. Evaporate the transferred layer at 60–80°C under forced air until dryness or constant volume. **Continued heating beyond this point may adversely affect test results**. This will take approximately **30 minutes** depending upon the evaporation equipment used.
12. Add 1 mL of ractopamine sample diluent buffer to the evaporated sample within **15 minutes** of the evaporation step. Vortex the sample for a minimum of **60 seconds** or until the sample is fully suspended.
13. Reconstituted samples can be directly tested using the ractopamine ELISA kit or stored frozen at -20°C for up to one week.

TEST PROCEDURE

1. Allow for all kit components, buffers, controls and samples to reach room temperature (20–23°C). Mix well by gentle inversion prior to use.
2. Determine the number of wells and amounts of reagents that will be required for immediate testing. Remove any excess strips from the strip holder. Store the strips in the original zipped bag. Dispense the necessary quantity of reconstituted enzyme. Return the unused strips and enzyme to the appropriate storage conditions.
3. Pipette 50 µL of the ready-to-use ractopamine controls into the wells (two wells each are recommended). Controls and samples should be added directly to the bottom of the wells.
4. Pipette 50 µL of each sample into the next available wells of the strip (minimum of two wells recommended).
5. Immediately add 100 µL of the reconstituted ractopamine enzyme to each well.
6. Using a timer, allow the reaction to occur at room temperature for exactly **10 minutes**.
7. Dump the solution from the wells, or aspirate using an automated washer. Wash the well by dispensing 350 µL of wash solution to each well then aspirating. Repeat the wash step two more times (three times total). Invert and firmly tamp the strip holder onto absorbent paper (such as paper towels) to remove the last traces of liquid from the wells.
   NOTE: A good wash step is critical to achieving low coefficients of variability and well-to-well reproducibility. Do not hesitate to aspirate on high or aggressively strike the strip holder on absorbent paper. Even small amounts of wash solution or bubbles remaining in the wells can affect results.
8. After ensuring the outside bottoms of the wells are clean and dry, immediately dispense the necessary volume of K-Blue Substrate from the storage bottle into an intermediate vessel, such as a clean reagent trough or a weigh boat, and transfer 100 µL of substrate to each well.
9. Allow the substrate to react at room temperature. This should take **10 minutes** for muscle samples and controls.
10. Stop the reaction by adding 100 µL of stop solution to each well containing substrate. **Do not dump the substrate**.
11. Using a microplate reader, determine the absorbance of each well at 450 nm (650 nm, 630 nm or 620 nm reference filter).

PERFORMANCE CHARACTERISTICS

Sensitivity (Detection Limit)

Sample Type	**Cut off Level** (ng/g or ppb)
Muscle Tissue	0.2*

*Cut off Level for USDA AMS Approved Verification Method in muscle tissue.

Specificity (Cross-Reactivity)

Analytes	**Cross-Reactivity** (%)
Ractopamine	100
Ritodrine	19.9
Fenoterol	0.5
Zilpaterol	0.05
Clenbuterol	<0.02
Salbutamol	<0.02
Terbutaline	<0.02
Groups E, J, K	<0.02

Group E: Acetylpromazine, Chlorpromazine, Desmethyldoxepin, Doxepin, Ethopropazine, Fluphenazine, Perphenazine, Phenelzine, Phenothiazine, Prochlorperazine, Promazine, Promethazine, Propiomazine, Propionylpromazine, Thioridazine, Trifluoperazine, Triflupromazine, Trimeprazine.

Group J: Triamcinolone, Desoxycorticosterone, Prednisolone, Hydrocortisone, Progesterone, Flumethasone, Betamethasone, Cortisone, Deoxycorticosterone, 19-Nortestosterone, Stanozolol, Boldenone, Dexamethasone, Corticosterone.

Group K: Sulfamethazine, Sulfadimethoxine, Sulfathiazole, Tylosin, Monensin, Gentamicin, Neomycin, Tetracycline, Penicillin G, Amoxicillin, Streptomycin, Erythromycin.

INTERPRETATION OF RESULTS

If the absorbance value of the sample is less than the cutoff control, then a concentration of ractopamine greater than the cutoff control is present in the sample.

If the absorbance value of the sample is greater than the cutoff control, then a concentration less than the cutoff control is present in the sample.

NOTE: In order for the test to be valid, wells with the negative control must have a bright yellow color (stopped) or a medium blue color (before stopping). Many factors influence the performance of this test. These may include the pH of the sample, temperature, endogenous interfering or cross-reacting compounds, the quality of the wash step, the type and calibration of equipment, and the skill of the analyst. Such factors should be considered when interpreting the results.

Neogen's ractopamine ELISA kit is intended for screening purposes only. Objective review and judgment must be used when interpreting results. Failure to follow kit instructions may lead to incorrect results.

CONFIRMATION

Confirmation of the results by a quantitative/official method, such as mass spectrometry, is required.

CUSTOMER SERVICE
Neogen Customer Assistance and Technical Services can be reached by calling 1-800-234-5333. Training on this product, and all Neogen test kits, is available.

SDS INFORMATION AVAILABLE
Safety data sheets (SDS) are available for this test kit, and all of Neogen's test kits, on Neogen's website at foodsafety.neogen.com, or by calling Neogen at 800/234-5333 or 517/372-9200.

TERMS AND CONDITIONS
For Neogen's full terms and conditions, please visit www.neogen.com/en/terms-and-conditions.

WARRANTY
Neogen Corporation makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. If any materials are defective, Neogen will provide a replacement of the product. Buyer assumes all risk and liability resulting from the use of this product. There is no warranty of merchantability of this product or of the fitness of the product for any purpose. Neogen shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.

OTHER RESIDUE DIAGNOSTICS KITS

9551	**Veratox for Chloramphenicol** — range 10–1,000 ppt, 96 wells
8416	**Veratox for Malachite Green** — range 1–4 ppb, 48 wells
DR021	**Veratox for Clenbuterol** — range 0.08–1.28 ppb, 96 wells
DR107	**Veratox for Florfenicol** — range 0.15–100 ppm, 96 wells
DR073	**Veratox for Avermectins** — range 6.4–300 ppb, 96 wells
DR081	**Veratox for Oxytetracycline** — range 3.6–324 ppb, 96 wells

For a complete list of products, visit foodsafety.neogen.com.

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