TECHNICAL PRODUCT INFORMATION

MaxSignal® Ractopamine ELISA Kit
Catalog # FOOD-1008-03

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MaxSignal® Ractopamine ELISA Verification Report

Introduction

Ractopamine has been banned for use as a livestock growth promoter in many regions and countries including Europe and China. To prevent ractopamine residues from entering the food chain, both producers and government surveillance agencies need technologies and methods that can provide rapid, accurate and reliable detection at specific sensitivities. MaxSignal® Ractopamine ELISA Kit enables government agencies, food manufacturers, as well as quality assurance organizations, to detect ractopamine as low as 0.1 ng/g or 0.1 ppb in a variety of sample types. In the current study, an improved sample preparation protocol was developed and validated for swine feed. In addition, other matrices listed in the product manual (offal, beef, pork, and urine samples) were validated.

The limit of detection (LOD) for different sample matrices was assessed on the MaxSignal® Ractopamine ELISA Kit (lot manufactured in May 2019). Samples were spiked with ractopamine standard spike at the Maximum Residue Limit (MRL) and a level above the MRL. Five replicates were tested per concentration from a single batch. LOD was defined as the mean plus three standard deviations of blank samples. Linearity of the assay was tested to assess performance.

Materials and Methods

Samples and Reagents

Naturally contaminated samples of known concentration were not available; therefore, artificially spiked samples were used. The samples were sourced locally. The spike solution was prepared from Sigma VETRANAL™ Ractopamine dihydrochloride standard (Cat No. 34918-100 mg, Sigma Aldrich). The nutritional composition of the feed sample tested is described in **Table 1**.

Nutrient	Level
Crude Protein, minimum	16.00%
Lysine, minimum	0.60%
Crude Fat, minimum	2.00%
Crude Fiber, maximum	18.00%
Calcium, minimum	0.75%
Calcium, maximum	1.25%
Phosphorus, minimum	0.40%

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Salt, minimum	0.50%
Salt, maximum	0.90%
Selenium, minimum	0.30 ppm
Zinc, minimum	150 ppm

Table 1. Table 1 describes the nutritional content of swine feed sample used in the validation study.

Sample Preparation

Offal, beef, pork, and urine samples were processed according to the extraction procedure listed in the product manual. Feed samples were processed as described below:

1. Weigh 2 ± 0.02 g of swine feed sample into 50-mL conical vial.
2. Weigh 0.4 ± 0.02 g of sodium chloride (NaCl), then add to the feed sample.
3. Add 5.0 mL of a 60:40 (% v/v) methanol/water solution and 1 mL of hexane to the sample.
4. Vortex the sample for 20 minutes, then centrifuge at 4,000 x g for 10 minutes at room temperature.
5. By carefully piercing through the upper hexane layer and any interphase that may have formed, transfer 100 µL of the middle, methanol layer into a new micro-centrifuge tube.
6. Add 100 µL of 0.5X RAC Sample Extraction Buffer (provided with each kit), then vortex for 1 minute.
7. Centrifuge at 13,000 x g for 2 minutes at room temperature.
8. Use the supernatant for ELISA.
   Dilution factor = 5

Experimental Design

To assess precision of the assay, five replicates of each blank and spike samples, spiked at MRL and a level above the MRL were used. Samples were extracted for ractopamine then tested and analyzed by ELISA following the assay protocol described in the manual (FOOD-1008-03).

Linearity of the assay was demonstrated by measuring repeated replicates of ractopamine dihydrochloride dissolved in methanol at six concentrations (0, 0.04, 0.1, 0.25, 0.6, 1.5 ppb). The assay was carried out and the results were plotted with spiked concentrations on the x-axis and absorbance at 450 nm on the y-axis.

Verification Criteria

Recovery of spiked samples should be in the range of 60-140% with an LOD < 0.5 ppb (regulatory limit) for meat and < 2 ppb for feed. For linearity verification, $R^2 > 0.96$ was desired.

Experimental Results

Linearity

The linearity of the assay exhibited results of $R^2 > 0.96$ for five replicate runs (**Figure 1**).

Precision and LOD

Precision experimental results are tabulated in **Table 2**. The results show an average recovery of 86% with recovery ranging from 66-98% and 79-91% for 2 and 4 ppb spikes with a confidence level of 100% within an acceptable range (60-140%) for feed samples. The LOD of feed samples was 0.875 ppb with a standard deviation of 0.049%. In offal (liver) samples, the average recovery was 104-116% with an LOD of 0.746 ppb. In beef muscle samples, the average recovery was 97-113% with an LOD of 0.08 ppb. In pork muscle sample, the average recovery was 83-89% with an LOD of 0.08 ppb. In urine samples, the average recovery was 104-109% with an LOD of 0.6 ppb.

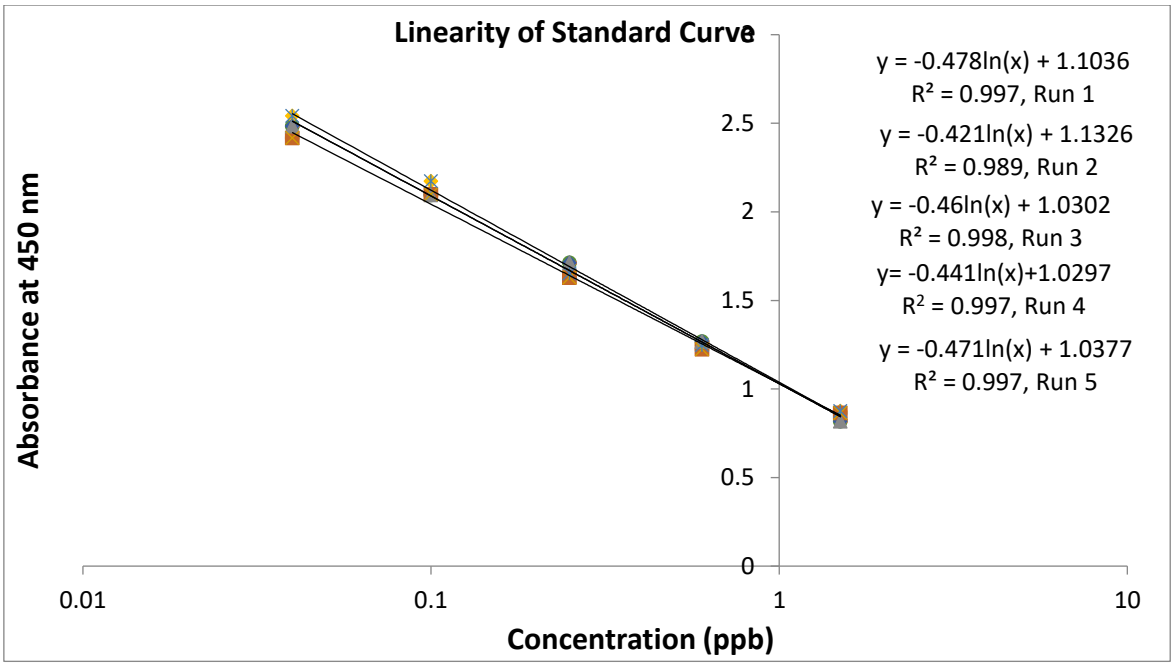

Figure 1. Figure demonstrating the linearity of the MaxSignal® Ractopamine ELISA.

Matrix	Spike (n = 5)	LOD (n = 5)	Standard Deviation (%)	Recovery	Mean
Swine Feed	2 ppb	0.875 ppb	0.049	66-98%	86%
	4 ppb	0.875 ppb		79-91%	86%
Offal (Liver)	0.5 ppb	0.746 ppb	0.038	73-122%	104%
	1 ppb	0.746 ppb		98-135%	116%
Beef	0.5 ppb	0.08 ppb	0.004	106-118%	113%
	1 ppb	0.08 ppb		79-114%	97%
Pork	0.5 ppb	0.083 ppb	0.0038	73-93%	83%
	1 ppb	0.083 ppb		85-103%	89%
Urine	1 ppb	0.601 ppb	0.085	83-130%	109%
	2 ppb	0.601 ppb		83-140%	104 %

Table 2. Summary of accuracy studies of MaxSignal® Ractopamine ELISA.

Conclusions

MaxSignal® Ractopamine ELISA Kit demonstrated excellent accuracy and precision for determination of ractopamine in swine feed samples and other matrices. Overall recoveries for all samples were within the acceptable range (60-140%) with a LOD below the Brazilian regulatory limit of 0.5 ppb for ractopamine in meat samples and 2 ppb for feed samples.