Version 26-04/24

User's Manual

Zilpaterol ELISA Kit

REF **DEIASL111**

Σ **96T**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

Zilpaterol ELISA Test Kit is a competitive enzyme immunoassay for the quantitative analysis of Zilpaterol in feed, tissue, urine, serum, plasma, milk. The unique features of the kit are:

1. Rapid (10 - 30 minutes), and organic reagent-free extraction method for various samples with high recovery (75 - 120%)
2. High sensitivity (0.05 ng/g or ppb)
3. A quick ELISA assay (less than 2 hours regardless of number of samples)
4. High reproducibility

General Description

Use of zilpaterol is approved for use as feed additive in some countries. Administering of this β-agonist during final fattening leads to an increased average daily gain of weight and improved meat/fat ratio. In the European Union and many other countries the use in food producing animals is illegal.

Principles of Testing

Zilpaterol ELISA test kit is based on the principle of competitive enzyme-linked reaction. The antibody containing zilpaterol has been coated on the microplate. For drug analysis, the sample and specific antibody are added to the wells of the plate together. If the sample contains a drug, it will compete for the antibody and inhibit the binding of the antigen to the drug antibody coated on the plate. Add enzyme-labeled secondary antibody to form a coating antibody-antigen-enzyme-labeled complex. After adding the substrate, the color intensity of the product is inversely proportional to the concentration of the drug in the sample.

Reagents And Materials Provided

Zilpaterol ELISA Test Kit has the capacity for 96 determinations or testing of 42 samples in duplicate (assuming 12 wells for standards). Return any unused microwells to the foil bag and reseal them with the desiccant provided in the original package.

1. Zilpaterol-coated Microtiter Plate: 1 x 96-well plate (8 wells x 12 strips)
2. Zilpaterol Standards:

   Negative control 1 mL

   0.05 ng/mL 1 mL

   0.15 ng/mL 1 mL

   0.45 ng/mL 1 mL

   1.35 ng/mL 1 mL

   4.05 ng/mL 1 mL

10 ng/mL(spiking, optional, red cap tube) 1 mL

3. Zilpaterol Antibody #1: 6 mL
4. HRP-Conjugated Antibody#2: 6 mL
5. 20×Wash Solution: 30 mL
6. Stop Buffer: 12 mL
7. TMB Substrate: 12 mL
8. 10×Sample Dilution Buffer: 25 mL
9. Sample Extraction Buffer A: 5 mL
10. Sample Extraction Buffer B: 5 mL

* If you are not planning to use the kit for over 3 months, store Zilpaterol Antibody #1 and HRP-Conjugated Antibody#2 working buffer at -20°C or in a freezer.

Materials Required But Not Supplied

1. Microtiter plate reader (450 nm)
2. Incubator
3. Tissue Mixer (e.g. Omni Tissue Master Homogenizer)
4. Vortex mixer (e.g. Gneie Vortex mixer from VWR)
5. 10, 20, 100 and 1000 µL pipettes
6. Multi-channel pipette: 50-300 µL (Optional)

Storage

Store the kit at 2-8°C The shelf life is 12 months when the kit is properly stored.

Specimen Collection And Preparation

Be sure samples are properly stored. In general, samples should be refrigerated at 2-4°C for no more than 1-2 days. Freeze samples to a minimum of -20°C if they need to be stored for a longer period. Frozen samples can be thawed at room temps (20 – 25°C / 68 – 77°F) or in a refrigerator before use.

Preparation of 1X Wash Solution

Mix 1 volume of the 20X Wash Solution with 19 volumes of distilled water.

Preparation of 1X Sample Dilution Buffer

Mix 1 volume of 10X Sample Dilution Buffer with 9 volumes of distilled water.

Feed

1. Homogenize a reasonable amount of feed sample by a suitable mixer.
2. Add 10 mL of 1x Sample Dilution Buffer to 1 g of the homogenized sample. Vortex for 3 minutes at a multi-tube vortexer or shaker.
3. Centrifuge at 4,000 x g for 5 minutes at room temperature .

4. Transfer 0.5 mL of upper layer to a new tube, add 0.5 ml of 1x Sample Dilution Buffer. Vortex at maximum speed for 30 seconds.
5. Use 50 µL of the sample for the assay.

Note: Dilution factor: 20

Tissue (muscle/liver)

1. Remove fat from muscle, liver. Homogenize the sample with a suitable mixer.
2. Add 3 mL of 1x Sample Dilution Buffer to 1g of the homogenized sample. Vortex for 3 minutes at maximum speed.
3. Centrifuge at 4,000 x g for 5 minutes at room temperature.
4. Use 50µL of the sample in the assay.

Note: Dilution factor: 4

Milk

1. Take 1 mL of milk sample, add 50µL of Sample Extraction Buffer A, vortex for 5 seconds, add 50µL of Sample Extraction Buffer B.
2. Vortex at maximum speed for 30 seconds.
3. Centrifuge at 4,000 x g for 5 minutes at room temperature .
4. Transfer 0.15 mL of upper layer to a new tube, add 0.45µL of 1x Sample Dilution Buffer. Vortex at maximum speed for 30 seconds.
5. Use 50µL of the sample in the assay.

Note: Dilution factor: 4.4

Urine/Serum/Plasma

1. Direct take 50µL of the sample per well for the assay.

   (If the sample is turbid, it is recommended to centrifuge at 4,000 x g for 5 minutes at room temperature and take 50 ul of supernatant for assay.)

Note: Dilution factor: 1

Reagent Preparation

IMPORTANT: All reagents should be brought up to room temperature before use (1 – 2 hours at 20 – 25°C / 68 – 77°F); Make sure you read “Precautions” section. Solutions should be prepared just prior to ELISA test. All reagents should be mixed by gently inverting or swirling prior to use. Prepare only the volumes that are needed for the number of wells being run. Do not return the reagents to the original stock tubes/bottles. It is recommended that disposable reservoirs be used when handling reagents to minimize the risk of contamination and is recommended.

Assay Procedure

Label the individual strips that will be used and aliquot reagents as the following example:

Component	Volume per Reaction	24 Reactions
Zilpaterol Antibody #1	50 µL	1.2 mL
HRP-Conjugated Antibody #2	50 µL	1.2 mL
1X Wash Solution	1.0 mL	24 mL
Stop Buffer	100 µL	2.4 mL
TMB Substrate	100 µL	2.4 mL

1. Add 50 µL of each Zilpaterol Standards in duplicate into different wells (Add standards to plate only in the order from low concentration to high concentration).
2. Add 50 µL of each sample in duplicate into different sample wells.
3. Add 50 µL of HRP-Conjugated Antibody #2 and 50 µL of Antibody #1, mix well by gently rocking the plate manually for 1 minute.
4. Incubate the plate for 30 minutes at room temperature (20 – 25°C / 68 – 77°F).
5. Wash the plate 4 times with 250 µL of 1X Wash Solution. After the last wash, invert the plate and gently tap the plate dry on paper towels (Perform the next step immediately after plate washings. Do not allow the plate to air dry between working steps).
6. Add 100 µL of TMB substrate. Time the reaction immediately after adding the substrate. Mix the solution by gently rocking the plate manually for 1 minute while incubating.
7. After incubating for 15 minutes at room temperature (20 – 25°C / 68 – 77°F), add 100 µL of Stop Buffer to stop the enzyme reaction.
8. Read the plate as soon as possible following the addition of Stop Buffer on a plate reader.

Calculation

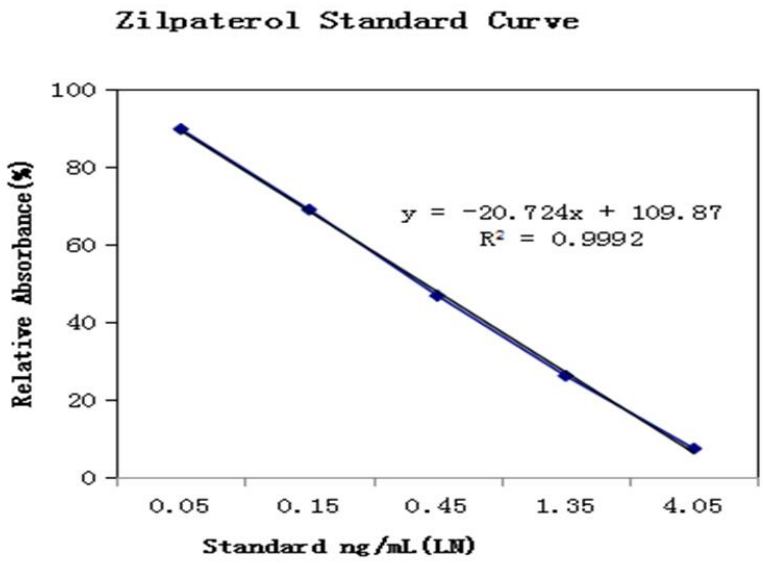
A standard curve can be constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ng/mL on a logarithmic curve.

$$\text{Relative absorbance (\%)} = \frac{\text{absorbance standard (or sample) x 100}}{\text{absorbance zero standard}}$$

Use the mean relative absorbance values for each sample to determine the corresponding concentration of the tested drug in ng/mL from the standard curve.

Typical Standard Curve

The following figure is a typical Zilpaterol standard curve:



Detection Limit

The limits of quantification are specified on the quality control certificate of the ELISA classic Zika Virus IgM. The linearity of dilution within this range has been demonstrated in comprehensive evaluation studies. If a sample shows a test result above the upper limit of quantification, the sample may be tested at a higher dilution. The resulting antibody activity must then be multiplied by the additional dilution factor.

Sensitivity

Sample Type	Detection Limit (ppb)
Feed	1
Tissue (muscle/liver)	0.2
Milk	0.22
Urine/Serum/Plasma	0.05

Specificity

Analytes	Cross-Reactivity (%)
Zilpaterol	100
Mabuterol	5.8
Mapenterol	4.2
Tolubuterol	2.8
Terbutaline	2.3
Cimbuterol	1.7
Salbutamol	1.0
Cimaterol	0.6
Pirbuterol	0.4
Isoprenaline	0.2

Precautions

1. The standards contain Zilpaterol. Handle with particular care.
2. Do not use the kit past the expiration date.
3. Do not intermix reagents from different kits or lots except for components with the same part No's within their expiration dates. ANTIBODIES AND PLATES ARE KIT-AND LOT-SPECIFIC. Make sure that the HRP Conjugate and Diluent are mixed in correct volumes.
4. Try to maintain a laboratory temperature of 20°–25°C (68°–77°F). Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation. Also, do not run assays in direct sunlight, as this may cause excessive heat and evaporation. Cold bench tops should be avoided by placing several layers of paper towel or some other insulate material under the assay plates during incubation.
5. Make sure you are using only distilled or deionized water since water quality is very important.
6. When pipetting samples or reagents into an empty microtiter plate, place the pipette tips in the lower corner of the well, making contact with the plastic.
7. Incubations of assay plates should be timed as precisely as possible. Be consistent when adding standards to the assay plate. Add your standards first and then your samples.
8. Add standards to plate only in the order from low concentration to high concentration, as this will minimize the risk of compromising the standard curve.
9. Always refrigerate plates in sealed bags with a desiccant to maintain stability. Prevent condensation from forming on plates by allowing them equilibrate to room temperature (20 – 25°C /68 – 77°F) while in the packaging.