Tribioscience

Salbutamol Fast ELISA (Catalog: TBS21141)

One-step-One-Hour ELISA For determination of salbutamol concentrations in serum, urine, tissue, and animal feed

Description

Salbutamol is a potent beta-2 agonist that can stimulate beta-2 receptors in mammals, which in turn leads to fat loss by allowing the body to release and burn more stored fat. It has been illegally used for decades in the veterinary world to increase the lean yield of livestock. Salbutamol can cause a multitude of adverse effects upon entering the human body, such as dizziness, nausea, diarrhea, agitation, muscle cramps, hypertension, and even acute myocardial infarction (heart attack). Therefore, it has been banned in many countries to be used on food-producing animals.

Tribioscience's Salbutamol ELISA Kit can provide a flexible, accurate, sensitive, and time-saving approach to determine if the salbutamol is present in animal feed, and urine and tissue samples of animals. It provides a vital tool to prevent the consumption of food tainted with this toxic chemical.

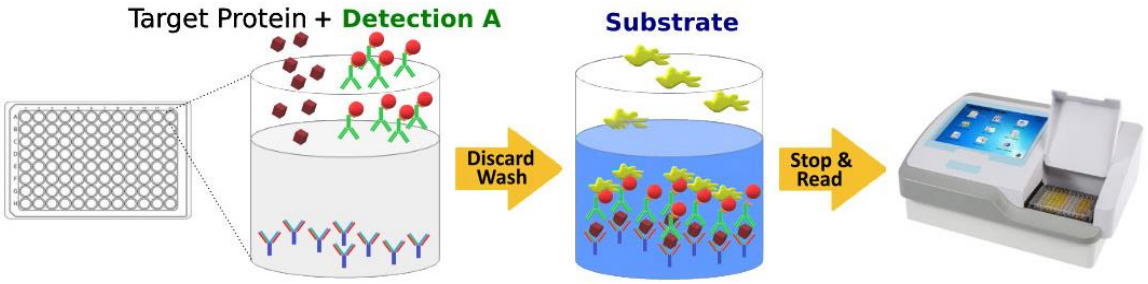
Intended Use

The Salbutamol Fast ELISA Kit utilizes competitive ELISA for the quantitative analysis of salbutamol in serum, plasma, urine, tissue and feed of farm animals. The limit of detection (LOD) of Salbutamol in ELISA Kit is 0.05ng/ml (0.05ppb).

Assay Principle

The Salbutamol Fast ELISA Kit is a competitive enzyme-labeled immunoassay (Fig. 1). The 96-well plate has been pre-coated with an anti-salbutamol antibody. During the assay, salbutamol standard or samples are added to test wells, followed by adding horse radish peroxidase (HRP)-salbutamol conjugate, which will compete with Salbutamol in standard or sample for binding to antibody during the 30-minute incubation. After plate wash, an ultra-sensitive TMB substrate is added to the wells leading to a colored product only in the presence of HRP, and optical density is inversely related to salbutamol concentrations in the samples. The accurate concentration of salbutamol can then be determined by using the standard curve constructed in the same run.

Fig. 1. Simple Procedures



KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Pre coated Microplate	TBS21141A	96 well microplate (12 strips of 8 wells) coated with an antibody specific for salbutamol.	Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
Salbutamol Standard	TBS21141B	100 µL of salbutamol (81 ng/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost the freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS21141C	80 µL of HRP- salbutamol conjugate (100x)	May be stored for up to 4 months at 2-8 °C.
Assay Diluent	TBS21141D	25 mL of a buffered protein base with preservatives.	
Wash Buffer	TBS3000W	12 mL of concentrated solution (10x).	
TMB Substrate	TBS3000T	10 mL of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 mL of 1 N sulfuric acid.	

Storage and Expiration Date
Storage: All components of the kit should be stored at 2-8℃. Expiration Date: This kit expires 12 months after the manufacturing date.

Safety Instructions
To receive complete safety information on this product, contact Tribioscience, Inc. and request Material Safety Data Sheets (MSDS).

Sample preparation
Meat (including fish and shrimp) sample:
1. Homogenize sample.
2. Weigh out 2.0 g homogenized sample.
3. Add 18 mL distilled water and 2 mL 1N HCl.
4. Homogenize and vortex for 3 min.
5. Centrifuge at 2000 rpm for 20 min at room temperature.
6. Take supernatant and adjust pH to 7-8 using 1N NaOH.
7. Centrifuge at 2000 rpm for 20 min at room temperature.
8. Use supernatant directly for ELISA assay.

Animal feed sample:
1. Weigh out 2.0 g homogenized sample.
2. Add 2ml ethyl acetate and mix by vortex thoroughly.
3. Centrifuge at 4000 rpm for 10 min at room temperature.
4. Take 1 mL supernatant.
5. Evaporate to dryness in a nitrogen evaporator.
6. Add 1 mL hexane to reconstitute.
7. Add 1 mL of sample diluent.
8. Vortex vigorously.
9. Centrifuge at 4000 rpm for 10 min at room temperature.
10. Discard upper layer, and transfer 50 µl bottom aqueous phase to ELISA plate.
11. Sample processed in this method has dilution factor of 2.

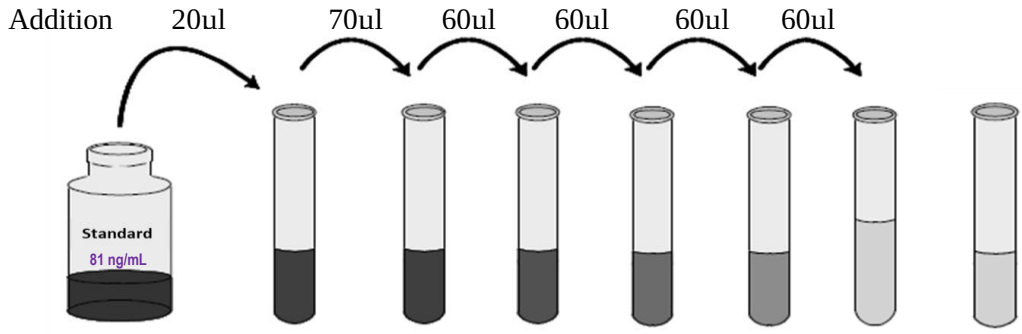
Urine samples:
1. Centrifuge at 4000 rpm for 5 min at room temperature.
2. Take supernatant and dilute 5-fold with sample diluent for ELISA assay.
3. Sample processed in this method has dilution factor of 5.

Assay Procedure
Equilibrate kit components at room temperature (20-25 °C) for at least 30 min prior to running the test, and thoroughly mix all liquid components before use.

Wash Buffer: Add 12 mL of Wash Buffer Concentrate(10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer.
HRP-Salbutamol Conjugate (Detection A): Prepare HRP working solution by diluting 1 part of HRP conjugate (100x) with 99 parts of Assay Diluent (1x) to HRP working solution (1x). For 1 plate, add 60 µL HRP-conjugate (100x) to 6 mL Assay Diluent.

Salbutamol Standard Preparation:
1. Label test tubes as #1 through #7.
2. Pipet 180 µL of 1x Assay Diluent into tube #1, and 140 µL into tubes #2 to #7 as diagram below (Fig.2).
3. Add 20 µL of Salbutamol Standard stock solution (81ng/mL) to tube #1(8.1ng/mL) and mix.
4. Make 3x serial dilutions of the standard using the Tube#1(8.1ng/mL standard solution) from Tube #2 through #6 with sequential transfer of 70 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 6 will be 8.1, 2.7, 0.9, 0.3, 0.1, and 0.03 ng/mL. Tube# 7 is Standard 0.

Fig.2 Diagram for salbumol standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7
Assay Buffer (µL)	180	140	140	140	140	140	140
Addition	Stock	Std1	Std2	Std3	Std4	Std5	
Addition Vol. (µL)	20	70	70	70	70	70	
Final Conc (ng/ml)	8.1	2.7	0.9	0.3	0.1	0.03	0

Assay Procedures:

1. Add 50 µL of standard, sample, or control per well.
2. Add 50 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 30min**.
3. Aspirate each well, and wash for 3 times by filling each well with 200 µL Wash Buffer. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µL of **TMB Substrate** to each well. Incubate **at RT for 10-20min** (*Protect from light*). The color becomes blue.
5. Add 50 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
6. Determine the optical density of each well within 20 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Quantitative Calculation of Salbutamol Concentration

A: Calculate B/B0: Dividing average absorbance of each standard and sample (B) by absorbance of the control of 0 ng/mL salbutamol concentration, B0) to obtain percentage absorbance.

Percentage absorbance (%) = 100%*(B/B_0).

B: Average absorbance of a standard or sample.

B_0: Average absorbance of 0 ng/mL control.

B: A standard curve is obtained by graphing the percentage absorbance of standards (Y axis) versus their corresponding concentration (X axis), and salbutamol concentration of can be read from this standard curve. Alternatively, salbutamol concentration in the samples can be calculated with regression equation correlating percentage absorbance to salbutamol concentration. Graphing software can also be used for quick analyses of samples.

Typical Data

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 3 is an example of typical Data.

Range of Standard Curve: 0 – 8.1 ng/ml

Assay Quantitative Range: 0.03 – 8.1 ng/ml

Assay Time: 50 min

Limit of Detection (LOD):
Meat 0.5 ppb
Feed 0.1 ppb
Urine 0.1 ppb

Recovery:
80-130%

Cross reactivity:
Clenbuterol 100%
Ractopamine <0.2%

Sensitivity (defined as the average of absorbance from 6 zero-standards minus 3 times of standard deviation):
0.05 ng/ml

Precision:
Intra-assay CV: <10%
Inter-assay CV: <12%

Precautions
1. Assay kit should be stored at 2-8℃ and avoid freezing conditions; unused test strips should be sealed in re-closable bag; colorless substrate is sensitive to light so prolonged exposure to light needs to be avoided.
2. Reagents should be brought to room temperature (20-25℃) prior to use. A room temperature of lower than 20℃ or failure to equilibrate reagents or samples to room temperature could result in low OD readings for all samples. All reagents should be put back into 2-8℃ storage immediately after use.
3. Do not use reagents beyond expiration date.

Technical Assistance
For ordering or technical assistance regarding this kit, or for additional information about Tribioscience products, please email: support@tribioscience.com or call (408) 498-0197, or 833-697-8998 (Toll Free).

Relative Product
Melamine rapid detection test strip (TBS11102)
Clenbuterol Rapid Test for Urine Samples (TBS11111)
Clenbuterol Rapid Test for Tissue Samples (TBS11112)
Chloramphenicol test strip (TBS11121)
Ractopamine rapid detection test strip (TBS11131)
Salbutamol rapid detection test strip (TBS11141)
Shiga Toxin (STX) Rapid Test Strip (TBS11151)
Vomitoxin / Deoxynivalenol (DON) Test Strip (TBS11156)
Ochratoxin A test strip (TBS11161)
Aflatoxin B1 test strip (TBS11166)
Zearalenone (ZEA) test strip (TBS11171)
Chloramphenicol Fast ELISA (TBS21121)
Clenbuterol Fast ELISA (TBS21111)
Total Aflatoxin Fast ELISA(TBS21131)
Ochratoxin-A Fast ELISA(TBS21133)

For research use only.