Data Sheet

Clenbuterol (CLE) ELISA Kit

Cat. #SG-4001

Size: 96 Wells

Sinogeneclon Co.,Ltd

Principle and Application

This kit adopts the method of indirect competitive enzyme-linked immunoassay (ELISA) to detect Clenbuterol (CLE) in the sample such as urine, tissue and feed. The kit is composed of Microtiter Plate coated with coupled antigens, HRP conjugate, antibodies, standards and other supporting reagents. During the detection, with adding standards or samples, the CLE in the samples will compete with the coupled antigens to combine with anti-CLE antibodies. After adding HRP conjugate, take coloration with TMB substrates. Absorbance value of the samples is a negative correlation with CLE content. Lastly, by comparing the obtained absorbance values with the standard curve, we can calculate the content of CLE content in the sample.

Storage conditions

- The kit shall be stored at 2-8 ℃. Avoid freezing.
- Shelf Life: 12 months. The date of manufacture is presented in the label of the box.

Technique Data

- Kit Sensitivity: 0.05ppb (ng/mL)
- Reactive Mode: 25℃, 30min～15min
- Detection Limits:

Sample	**Detection Limits**
Urine	0.1ppb
Tissue	0.2ppb
Feed	1ppb
Pork liver	0.5ppb

- Cross-reaction Rate:

Clenbuterol ……....100%

Terbutalin …….. <1%

Mabuterol……..…… <1%

Brombuterol……<1%

Salbutamol…….. <1%

Ractopamine……....<1%

- Sample Recovery Rate:

Sample	**Recovery Rate**
Urine	95±10%
Tissue, feed, pork liver	85±15%

Composition of the Kit

Reagent	**Specification**
Microtiter Plate	8wells× 12strips
Standard: 0ppb, 0.05ppb, 0.15ppb, 0.45ppb, 1.35ppb, 4.05ppb (black cap)	1.0mL each
High standard (red cap)	100ppb
Antibody solution (blue cap)	1×5.5mL
HRP conjugate (red cap)	1×5.5mL
Substrate Reagent A (white cap)	1×6mL
Substrate Reagent B (black cap)	1×6mL
Stop Solution (yellow cap)	1×6mL
Concentrated Wash Buffer (20×)(white cap)	1×40mL
Concentrated Reconstitution Buffer (10×)(yellow cap)	1×50mL
Instruction	1
Adhesive Membrane	1
Sealed bag	1

Materials Required but Not Supplied

- **Equipment:** microplate reader, printer, grinder (for homogenizing solid samples), nitrogen evaporator, vortex mixer (for shake and mix), centrifuge, graduated transfer pipette, and balance with a division value of 0.01 g, constant temperature device;
- **Micropipette:** single-channel (20-200μL, 100-1000μL), and multi-channel 300μL;
- **Reagents:** Sodium Hydroxide, Ethyl Acetate, Concentrated Hydrochloric Acid, methanol, Anhydrous Sodium Carbonate.

Experimental preparation

Restore all reagents and samples to room temperature (adjust to around 25°C) for more than 30 min before use. This is a crucial step to ensure there is no precipitation in the reagents.

Please note that the labware must be clean. Use disposable pipette tips to avoid contamination of interference results.

◆ **Solution preparation:**

Solution 1: Reconstitution Buffer

Dilute the Concentrated Reconstitution Buffer (10×）10 times (Concentrated Reconstitution Buffer (10×) /Deionized water= 1:9). It can be stored for one month at 4°C.

Solution 2: Working Wash Buffer

Dilute the concentrated wash buffer (20×) by a factor of 20 (Concentrated wash buffer/Deionized water= 1: 19).

Solution 3: Sample Extraction Solution

70% Methanol solution, (Methanol/Deionized water= 7: 3)

Solution 4: 10% Sodium Carbonate Solution

Dissolve 50 g of anhydrous sodium carbonate in deionized water and dilute to a total volume of 450 mL.

Solution 5: 0.1M Hydrochloric Acid Solution

Add 860 μL of concentrated hydrochloric acid to deionized water and dilute to a total volume of 100 mL.

Solution 6: 2M Sodium Hydroxide Solution

Dissolve 8 g of sodium hydroxide in deionized water and dilute to a total volume of 100 mL.

◆ **Sample pretreatment steps:**

1. Urine treatment.

1) Take 1mL of clear urine sample (if the urine is turbid, filter it or centrifuge at 4000r/min for 5 minutes at room temperature to obtain a clear sample) in a 10mL centrifuge tube. Add 1mL of **reconstitution buffer (solution 1)**, mix and shake for 30 seconds, then take 50μL for analysis. Samples not in use should be stored frozen.

Dilution times of the sample:2 Detection limits: 0.1ppb

2. Tissue treatment.

1) Weigh 1g ± 0.05g of homogenized tissue sample, add 3mL of **reconstitution buffer (Solution 1)**, shake thoroughly for 2 minutes, and centrifuge at 4000r/min at room temperature for 10 minutes. (If the tissue sample has a high fat content, place it in an 85℃ water bath for 10 minutes after shaking, then centrifuge.) Take 50μL of the supernatant for analysis.

Dilution times of the sample:4 Detection limits:0.2ppb

3. Animal feed treatment.

1) Weigh 1g ± 0.05g of ground feed sample into a 15mL centrifuge tube, add 4mL of **sample extraction solution (solution 3)**, shake thoroughly for 2 minutes, and centrifuge at 4000r/min at room temperature for 10 minutes.

2) Transfer 0.1mL of the supernatant to a 1.5mL centrifuge tube, add 0.4mL of **reconstitution buffer (solution 1)**, shake thoroughly for 30 seconds, and take 50μL for analysis.

Dilution times of the sample:20 Detection limits: 1ppb

4. Pork liver treatment.

1) Weigh 1g ± 0.05g of homogenized pig liver sample into a 15mL centrifuge tube, add 1mL of **10% sodium carbonate solution (solution 4)**, shake thoroughly for 2 minutes, add 4mL of ethyl acetate, shake thoroughly for 5 minutes, and centrifuge at 4000r/min at room temperature for 10 minutes

2) Transfer 2 mL of the supernatant to a 15 mL centrifuge tube, add 1 mL of **0.1M hydrochloric acid solution (Solution 5)**, vortex for 2 minutes to mix thoroughly, and then centrifuge at room temperature at 4000 rpm or higher for 5 minutes.

3) Transfer 0.1mL of the lower clear layer to a 1.5mL centrifuge tube, add 0.4mL of **reconstitution buffer (solution 1)**, adjust the pH to around 7 using **2M sodium hydroxide solution (solution 6)**, and take 50μL for analysis.

Dilution times of the sample:10 Detection limits: 0.5ppb

ELISA procedure

Place all reagents and samples to room temperature (adjust to around 25°C) for 30min. Gently shake the reagent bottles before use.

Take out the frame of the microplate along with the required number of wells. Then place the unused microplate wells into the sealed bag with the desiccant provided. Store the remaining kit in the refrigerator at 2-8°C.

Step 1: Number: Number the wells in sequence corresponding to the samples and standard, make 2-well parallel trials for each sample and standard, and record their locations.

Step 2: Incubation: Add 50μL of **standard or sample** into each numbered well, then add

50μL of **HRP conjugate** per well. Next, add 50μL of **antibody solution** per well. Finally, cover the Microtiter Plate with the **adhesive membrane**, shake gently by hand (or use a microplate shaker) for 5s and incubate for 30 min at 25℃.

Step 3: Washing: Uncover the adhesive membrane carefully, remove the liquid, pipette 350μL of **Working Wash Buffer (Solution 2)** to every well, let stand for 30 seconds then drain, repeat 5 times. Invert the plate and tap it against a thick absorbent paper (or lint-free cloth), with a soft towel placed underneath. (Bubbles that are not removed after patting dry can be punctured with a clean pipette tip).

Step 4: Color: Add 50μL of **Substrate Reagent A** to each well, then add 50μL of **Substrate Reagent B** per well. Shake gently by hand (or use a microplate shaker) for 5s, and allow to react for 15min at 25℃ in the dark. (The reaction can be extended appropriately if the blue colour is too pale.)

Step 5: Stop the reaction: Pipette 50μL of **Stop Solution** to each well, shake gently by hand (or use a microplate shaker) for 5s.

The reaction would be stopped.

Step 6: Calculate: Determine the Optical Density (OD value; absorbance value) at 450nm (Reference wavelength 630nm) with a microplate reader. Finish this step within 10min after stop the reaction.

Interpretation of result

◆ **Calculate the percentage of absorbance value**

$$\textbf{Percentage of absorbance value}(\%) = \frac{A}{A0} \times 100\%$$

A—the average OD value of the sample or the standard;

A0—the average OD value of the 0ppb standard.

It is used to calculate the percentage absorbance of a standard or sample.

◆ **Draw the standard curve and calculate**

- Take absorbance percentage (A/A0) of standards as Y-axis and the corresponding log of standards concentration (ppb) as X-axis.
- Draw the standard semi-log curves with X-axis and Y-axis.
- Take absorbance percentage of samples substitute into standard curve, then can get the corresponding concentration from standard curve. Last, **the resulting concentration values multiplied by the corresponding dilution times is the actual concentration of CLE of samples.**

  If professional analysis software of the kit is used for calculation, it is more convenient for accurate and rapid analysis of a large number of samples.

Attention

- Before test, the reagents and samples should be balanced to room temperature (25℃). If below 25℃, it will lead to all the

  standard OD value on the low side.
- In the washing process, dry wells may result in non-linear standard curves and undesirable reproducibility. Therefore, proceed to the next step immediately after washing.
- The reproducibility is largely determined by consistency of washing step. Please mix uniformly and wash thoroughly.
- During the incubation, cover microplates with adhesive membrane to avoid light.
- Do not use kits that are overdue. Do not mix reagents with those from other lots.
- Substrate Reagent A/B is colorless. If not, please discard.
- If absorbance value of 0ppb is below 0.5（A450nm< 0.5 ）, it means that the reagent may be metamorphic.
- Stop solution is corrosives. Please avoid contacting with skin.
- **As the OD values of the standard curve may vary according to the conditions of actual assay performance (e.g. operator, pipetting technique, washing technique**

or temperature effects), the operator should establish a standard curve for each test.

- **For the mentioned sample, fast and efficient extraction methods are included in the kit description. Please consult technical support for the applicability if other sample need to be tested.**
- The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS can be used for quantitative confirmation.