



Abkem Iberia

KIT PRESENTATION

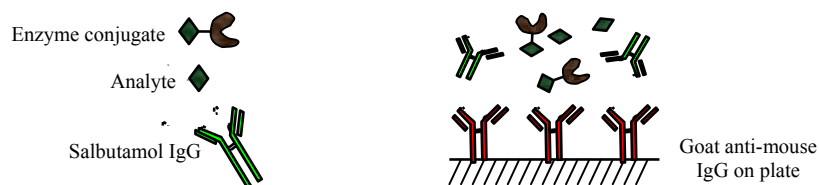
β_2 Agonists: **Clenbuterol** and **Salbutamol** are used as therapeutic agents in veterinary medicine. At higher dosage levels these compounds may also be illegally used as repartitioning agents in meat-producing animals. Residues of these β_2 agonists could present a potential risk for the health of individuals consuming illegally treated animal-derived foods. **β_2 agonists DiagnoKit™** allows to perform quality control of animal tissues and biological fluids to check for the possible illegal use of **β_2 agonists** as growth promoters.

Kit Description:

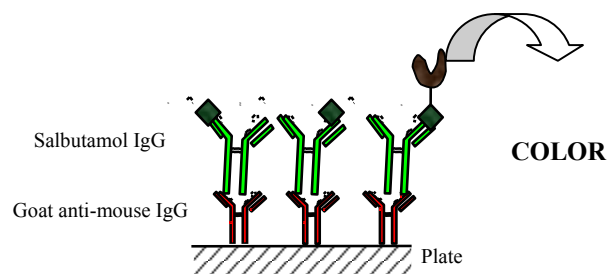
- Indirect enzyme-immunoassay for the quantitative analysis of β_2 Agonists.
- Suggested use: cattle food, urine and serum samples.

Test Principle:

The test is based on a competitive binding of Clenbuterol enzyme conjugate and free Clenbuterol or Salbutamol to a specific Salbutamol antibody.



Antibody-antigen complexes are specifically bound to sheep anti-rabbit immobilized IgG on microtiter wells. Color development, following the addition of substrate is directly correlated to the concentration of the analyte in the tested samples.





Kit Content provided:

- Calculation diskette (MS Excel)
- 1 microtiter plate (12 strips of 8 wells) coated with purified goat anti-mouse IgG.
- Salbutamol standard (1000 ng, Lyophilized)
- Salbutamol antibodies (Lyophilized)
- Clenbuterol-HRP conjugate (Lyophilized)
- Washing buffer (dry powder)
- Dilution buffer (dry powder)
- Substrate Solution A, containing TMB (15mL)
- Substrate Solution B, containing H₂O₂* (1mL)
- Stopping reagent, containing H₂SO₄* (10mL)

* Corrosive, use with care

Materials required, not provided:

- ❖ Precision adjustable pipet and a 12 or 8 channel multipipet able to deliver 200µL
- ❖ Plate shaker suitable for enzyme immunoassay
- ❖ Plate reader with 450 nm interference filter
- ❖ Test tubes
- ❖ 500 mL squeeze bottle
- ❖ De-ionized water
- ❖ Timer

Additional useful items:

- ❖ Washing buffer (Cat.# D0310)
- ❖ Dilution buffer (Cat.#D0320)
- ❖ Extraction buffer (Cat.#D330)

Safety Precautions:

Items included in this kit are to be used by suitable qualified laboratory personnel, under proper laboratory working conditions. Handle all reagents and antibody in accordance with local safety procedures. Avoid any skin contact with stop solution and substrate B, in case of contact wash very well with water. MSDS (Material Safety Data Sheets) available upon request.

Procedural notes:

Store the kit at 2-8 °C. Before start the assay all reagents should be equilibrated at room temperature. Return all reagents to 2-8°C immediately after use. Do not interchange reagents between kits of different lot numbers. Do not use reagents beyond the expiration date of the kit. Substrate solution is light sensitive. Avoid exposure to direct light, and avoid contact with metal, which can cause colour development. A dark blue colour developed by the substrate solution after preparation is indicative of contamination. Sample extracts can be stored at 2-8°C for seven days and at -20°C for several months. Samples diluted in dilution buffer can be stored at 2-8°C only for three days.



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Preparation of Reagents

Prepare fresh diluted reagents, just prior to use

Washing Buffer:

Washing buffer is lyophilized and equivalent to 200 mL (100 mL in each bottle). Dissolve each bottle with dry powder in 25 mL of purified water to obtain washing buffer **4 times more** concentrated. Then, dilute 1:4 with purified water the necessary volume.

Dilution Buffer:

Dilution buffer is lyophilized and equivalent to 15 mL. Dissolve the dry powder in 15 mL of purified water.

Standard solutions:

Dilute in 1 mL of dilution buffer for obtaining a **Salbutamol Standard Solution with 1000 ng/mL**.
Preparation of Standards:

Standard 1 →50 ng/mL:

50 μ L of Salbutamol Standard Solution of 1000 ng/mL + 950 μ L of **dilution buffer** to obtain 1 mL of Standard 1.

Standard 2 →40 ng/mL

40 μ L of Salbutamol Standard Solution of 1000 ng/mL + 960 μ L of **dilution buffer** to obtain 1 mL of Standard 2.

Standard 3 →30 ng/mL

30 μ L of Salbutamol Standard Solution of 1000 ng/mL + 970 μ L of **dilution buffer** to obtain 1 mL of Standard 3.

Standard 4 →20 ng/mL

20 μ L of Salbutamol Standard Solution of 1000 ng/mL + 980 μ L of **dilution buffer** to obtain 1 mL of Standard 4.

Standard 5 →10 ng/mL

400 μ L of Standard 4 of 20 ng/mL + 400 μ L of **dilution buffer** to obtain 800 μ L of Standard 5.

Standard 6 →5 ng/mL

400 μ L of Standard 5 of 10 ng/mL + 400 μ L of **dilution buffer** to obtain 800 μ L of Standard 6.

Note: Standards are prepared 4X more concentrated than those to be obtained on plate.

Salbutamol antibody:

Reconstitute the lyophilized antibody with 5 mL of purified water, mix and store in the freezer after use for guaranteed conservation.



Clenbuterol-HRP conjugate:

Reconstitute the lyophilized enzyme conjugate with 2500 μL of purified water. Store between 4-8°C after use. Stable for one week after reconstitution.

Substrate solution

This solution should be prepared immediately prior to its use, by mixing the Substrate Solution A & B in the following proportion: 10 mL of Substrate Solution A + 5 μL of Substrate Solution B. Prepare only the needed amount of this solution. (e.g.: for 3 strips you should prepare a total of 3 mL)

Stopping solution:

Ready to use.

Test Procedure

1. Rinsing protocol: Fill each well with 100 μL of washing buffer, using a precision pipet, turn the plate upside down and empty wells. The rinsing cycle should be carried out 5 times. Remove residual liquid by tapping the plate upside down on an absorbent paper.
2. Using a precision pipet transfer 25 μL of each standard solution for calibration into the wells as follows:

A1, A2 Blank to evaluate non specific binding of reagents: 25 μL of dilution buffer, no standard
B1, B2 Maximum OD Blank: 25 μL of dilution buffer, no standard
C1, C2 25 μL of the 50 ng/ml standard (Std-1)
D1, D2 25 μL of the 40 ng/ml standard (Std-2)
E1, E2 25 μL of the 30 ng/ml standard (Std-3)
F1, F2 25 μL of the 20 ng/ml standard (Std-4)
G1, G2 25 μL of the 10 ng/ml standard (Std-5)
H1, H2 25 μL of the 5 ng/ml standard (Std-6)

3. Using a precision pipet transfer 25 μL of each diluted unknown sample extract into assigned well.

Addition of the enzyme conjugate

4. Add 25 μL of the diluted Clenbuterol-HRP conjugate into each well (no. A1,A2 to H1,H2 for standards and each unknown sample well)

Addition of the antibody

5. Add 50 μL of **dilution buffer** to well A1 and A2 for the evaluation of non specific binding.
6. Add 50 μL of the diluted Salbutamol antibody into every well, except in the well no.A1, A2 (non specific).
7. Incubate plate at 2-8°C for 2 hours.
8. Empty the plate by inverting it over the sink then wash each well 5 times (see step no.1)
9. Add 100 μL of the substrate solution TMB/H₂O₂ to each well. Mix thoroughly and incubate for 30 minutes in the dark at room temperature.
10. Add 50 μL of the stopping solution to each well. Mix and incubate for 10 minutes in the dark at room temperature.
11. Take measurement of the absorbance with a plate reader at 450nm.



Results

An example of data processing is presented under a Micro Soft excel format and provided in the attached disk. A calculation table allows you to tabulate the mean O.D. for a duplicate or triplicate run or of standard solution. Resulting graph will be suggested.

Data is treated so as the mean value of the absorbance (450nm) readings obtained for the standards and the samples are reported to the absorbance value of the zero standard.

$$\left[\frac{\text{Absorbance standard (or sample)} - \text{Absorbance of NS}}{\text{Absorbance zero standard} - \text{absorbance of NS.}} \right] \times 100 = \% \text{ B/Bo}$$

NS = non specific

Maximum OD Blank = zero standard

A calibration curve can be obtained using the calculated % B/Bo value for each standard versus the log of the corresponding Clenbuterol concentration (in ng/ml).

Take the B/Bo (%) value for each sample and interpolate the corresponding concentration from the calibration curve. The linear transformation of this calibration curve may be obtained by plotting, logit (%B/Bo) versus $\ln C$, where:

$$\text{logit } \% \text{ B/Bo} = \ln \left[\frac{\% \text{B/Bo}}{100 - \% \text{B/Bo}} \right]$$

➤ see provided disk, MS-Excel file

In order to obtain the Clenbuterol concentration in ng/ml contained in a sample, use one of the linear ranges of the calibration curve of your choice. The determined value must be further multiplied by the corresponding dilution factor. This is based on the assumption that the recovery after extraction is 100%.



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Suggested extraction of samples

Tissue extraction:

- ❖ Weigh 5 g of tissue in a 50 mL tube,
- ❖ Add 15 mL acetonitrile, 5 mL HCl 1M and 10 mL petroleum ether,
- ❖ Homogenize for 15 minutes by agitation,
- ❖ Centrifuge at 4000 RPM for 10 minutes,
- ❖ Eliminate the petroleum ether by aspiration and decant the liquid phase,
- ❖ Reduce the liquid phase at approximately 5 mL at 60°C under a nitrogen stream,
- ❖ Add 3 mL NaOH 5N, 20 mL of dichloromethane and vortex for 15 minutes,
- ❖ Centrifuge at 4000 RPM for 10 minutes,
- ❖ Eliminate the supernatant (acetonitrile/HCl) and reduce the dichloromethane phase to dryness,
- ❖ Dissolve the residue into 500 µL of ethanol and 4.5 mL of the dilution buffer.

Urine quick test

50µL urine can be applied after centrifugation and dilution 5 times with dilution buffer.

TECHNICAL SUPPORT:

Please write to:
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