

**INDUSTRY STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

SN/T 5119-2019

**Determination of neomycin residues in edible animal for import
and export - ELISA and LC-MS/MS**

进出口食用动物中新霉素药物残留测定 酶联免疫吸附法和液相色谱-质谱/质谱法
(English Translation)

Issued on: September 3, 2019

Implemented on: March 1, 2020

Issued by: General Administration of Customs

Table of Contents

Foreword

1 Scope

2 Normative references

3 Principle

4.1 Water. secondary water specified in GB/T 6682-2008

4.2 Carbodiimide. Analytical grade

4.3 Ovalbumin

4.4 Bovine serum albumin

4.5 Dialysis Bag

4.6 Coating buffer

4.7 Washing buffer

4.8 Blocking buffer

4.9 Dilution buffer

4.10 Sample Extraction Solution

4.11 Diluent of horseradish peroxidase labeled conjugate

4.12 Substrate solution

4.13 Developer solution

4.14 Stop solution

4.15 Antibodies

4.16 HRP conjugate

4.17 Ovalbumin-coated neomycin polystyrene micro 96-well reaction plate

5 Instruments and equipment

6 Measurement steps

6.1 Sample preparation and storage

6.2 Extraction

6.3 Enzyme-linked immunoassay

6.4 Blank test

6.5 Quality control test

7 Results calculation

7.1 Drawing of standard curve

7.2 Calculation of percent absorbance

7.3 Calculation of the content of the target substance in the test solution

- 8 Method performance indicators
 - 8.1 Sensitivity
 - 8.2 Recovery rate
 - 8.3 Precision
- 9 Reagent materials
- 10 Instruments and equipment
- 11 Measurement steps
 - 11.1 Extraction
 - 11.2 Purification
 - 11.3 Determination
 - 11.4 Blank test
- 12 Calculation and presentation of results
- 13 Determination of low limit, recovery and precision
 - 13.1 Lower limit of determination
 - 13.2 Recovery and precision

Foreword

This standard was drafted in accordance with the rules given in GB/T 1.1-2009.

Please note that some content of this document may involve patents, and the issuing authority of this document is not responsible for identifying these patents.

This standard is proposed and managed by the General Administration of Customs of the People's Republic of China.

The main drafting organization of this standard. Qingdao Customs of the People's Republic of China.

1 Scope

This standard specifies the enzyme-linked immunosorbent assay method and liquid chromatography for neomycin residues in the plasma and urine of food animals for import and export.

Spectral determination method.

This standard is applicable to the rapid detection of neomycin residues in the blood and urine of pigs, cattle and sheep for import and export of food animals.

2 Normative references

The following documents are essential for the application of this document. For dated references, only the dated version applies to this document.

file. For undated references, the latest version (including all amendments) applies to this document.

GB/T 6682-2008 Analytical laboratory water specifications and test methods Enzyme-linked immunosorbent assay

3 Principle

Based on a competitive enzyme-linked immune response, neomycin-containing antigens are coated on microplates. For drug analysis, samples are co-existed with specific primary antibodies the same is added to the plate hole. If the sample contains a drug, it will compete with the primary antibody, inhibiting the antibody from binding to the drug antigen coated on the plate. Add enzyme Label the secondary antibody to form a coated antigen-antibody-enzyme-labeled secondary antibody complex. After adding the substrate, the color of the product is proportional to the concentration of the drug in the sample.

Inversely.

4 Reagents and materials

4.13 Developer solution

SN/T 5111-2019????, ????, ????,?

4.17 Ovalbumin-coated neomycin polystyrene micro 96-well reaction plate

4.18 Standard. neomycin sulfate, purity 90%.

4.19 Standard stock solution. Weigh the standard accurately, convert it to 10.0 mg of the target compound, dissolve it with an appropriate amount of water, and make up to volume with water.

10mL, mix well. Store below -18 ° C in the dark, with a validity of 12 months.

4.20 Standard intermediate solution. Dilute the standard stock solution with water to 10 mg/L, and store it in the dark below 4 ° C. The validity period is 6 months.

4.21 Standard working solution. Dilute the standard intermediate solution to a working concentration of 1.5 µg/L, 3 µg/L, 6 µg/L, 12 µg/L, 36 µg/L with dilution buffer.

Note: 4 For the preparation of various liquids involved in reagents and materials, see Appendix A.

5 Instruments and equipment

5.1

Analytical balance with a sensitivity of 0.0001g.

5.2

Microplate reader with 450nm filter.

5.3

Centrifuge. The number of revolutions is 8000r/min.

5.4

Ultrasound generator.

5.5

Micropipettes. 20 μ L, 50 μ L, 100 μ L for single channel and 50-350 μ L for multiple channels.

5.6 pH meter. pH measurement range 0-14.

5.7

Vortex mixer.

5.8

Oscillator.

6.1.1 Sample preparation

10 ml of animal blood was collected in anticoagulant test tubes containing sodium heparin, and 50 ml of fresh urine was placed in clean containers.

Two aliquots were sealed as samples and marked. During sample preparation, samples should be protected from contamination or residues.

Variety.

6.1.2 Sample preservation

The sample can be stored at 4 ° C for 3-4 days; if it is stored for a long time, the sample should be placed below -18 ° C.

6.2 Extraction

Take 50 μ L of sample, add 1.2 mL of sample extract, mix well, centrifuge at 4000 x g for 5 min, and use the supernatant for detection.