Streptomycin ELISA Kit

Enzyme Immunoassay for the quantitative determination of Streptomycin in food

Catalog number: ARG80814

Package: 96 wells

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INTRODUCTION

Streptomycin consists of three components, which are linked together by glycoside bonds, and it be-longs to the group of the aminoglycoside antibiotics. Streptomycin is naturally produced by the actinobacterium Streptomyces griseus, and its activity is directed against gram-negative bacteria and the tuber-cle bacillus. Therapeutically it is used in the case of streptococcal and enterococcal enteritis. Because of its side effects (damage of equilibrium and auditory nerve, as well as the kidney) it is rarely used in the human treatment, but has an application in the ve-te-rinary area. After the treatment of mastitis in bree-ding animals, elevated streptomycin values were also measured in liver, kidney, muscle and milk. The maximum permissible values are in these cases: 500 µg/kg, 1000 µg/kg, 500 µg/kg and 200 µg/kg. Another application of the antibiotic streptomycin under the brand name of Plantomycin is the treatment of the illness of fruit trees called fire blight. In order to reduce the transmission to humans, maximum permissible val-ues were defined in the European Union. The Ger-man critical value for streptomycin in honey according to the RHmV regulation is at the moment 20 µg/kg.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. An antibody directed against mouse immunoglobulins is coated on the surface of a microtiter plate. Streptomycin containing samples or standards and an antibody directed against streptomycin are given into the wells of the microtiter plate. The streptomycin contained in samples or standards will bind

to the antibody which reacts with the anti-mouse antibody coated onto the microtiter plate. After 30 minutes incubation at room temperature a streptomycin-peroxidase conjugate is added into the wells without a preceding washing step to saturate free antibody binding sites. After additional 15 minutes incubation at room temperature the wells are washed with diluted washing solution to remove unbound material. A substrate solution is added and incubated for 15 minutes, resulting in the development of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured at 450 nm. The concentration of streptomycin is indirectly proportional to the color intensity of the test sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C
Anti-Streptomycin antibody	6 ml (ready to use)	4°C
HRP-Streptomycin Conjugate	6 ml (ready to use)	4°C
Standard 0-5 (0, 2, 5, 20, 50, 200 ng/ml)	6 X 1 ml (ready to use)	4°C
Sample dilution buffer	2 X 60 ml (ready to use)	4°C
10x Wash Buffer	60 ml	4°C
TMB substrate	15 ml (ready to use)	4°C (Protect from light)
STOP solution	15 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- 0.01 M PBS (add 8.77 g of NaCl, 0.7 g of $NaH_2PO_4 \bullet 2H_2O$, 2.9 g of $Na_2HPO_4 \bullet 2H_2O$ in 800 ml of distilled water, Adjust solution to pH 7.3. Then add distilled water until volume is 1 L)
- Extraction buffer (2.0 g of heptanesulfonic acid sodium salt, 1.9 g of $Na_3PO_4 \bullet 12H_2O$ add 200 mL of double distilled water, adjust pH 2.0 with o-phosphoric acid)
- Methanol (100%)
- Carrez I solution: Dilute 15 g of potassium hexacyanoferrate(II) trihydrate in distilled water and adjust volume to 100 mL. (150 g/L))
- Carrez II solution: Dilute 30 g of Zinc sulfate-7-hydrate in distilled water and adjust the volume to 100 mL. (300 g/L)
- C18 SPE column (optional)
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- If crystals are observed in the 10X Wash buffer and Sample dilution buffer, warm to RT or 37°C for 15 min or until the crystals are

completely dissolved.

- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
- Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
- Use a separate disposable tip for each specimen to prevent cross-contamination.
- All specimens and standards should be run at the same time, so that all conditions of testing are the same.
- Do not mix components from different batches.
- Do not use reagents after expiration date.
- Check both precision and accuracy of the laboratory equipment used during the procedure (micropipets, ELISA reader etc.)
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

Honey (Screening Method)

1. Dissolve 2 g honey sample in 10 ml distilled water.
2. Further dilute this extract 1:4 with Sample dilution buffer.
3. Sample dilution factor: **F=20**

Honey (Sensitive Method; C18 SPE)

1. Fill 1 g honey sample up to 10 mL extraction buffer. Clear sample by centrifugation (10 minutes at 3000 g).
2. Rinse a C18 SPE column with 2 mL methanol (100%) followed by 2 ml double distilled water.
3. Push 5 mL sample slowly through the column (ca. 1 ml/min).
4. Rinse column with 3 mL double distilled water.
5. Dry column 2 minutes by air or nitrogen stream.
6. Apply 1 ml methanol (100%) onto the column and elute sample (ca. 1 ml/min).
7. Evaporate eluate in an air or nitrogen stream at 50-60°C.
8. Dissolve the residue in 2 ml Sample dilution buffer and test this sample in the ELISA.
9. Sample dilution factor: **F=4**

Shrimps

1. Mill and homogenize sample with an appropriate device (mixer, ultra-turrax).
2. Mix 1 g sample with 4 ml 0.01 M PBS and agitate vigorously for 30 minutes.

3. Centrifuge for 10 minutes at 3000 g.
4. Dilute the clear supernatant 1:4 in Sample dilution buffer and test this sample in the ELISA.
5. Sample dilution factor: **F=16**

Meat

1. Mill and homogenize sample with an appropriate device (mixer, ultra-turrax).
2. Mix 1 g sample with 4 ml 0.01 M PBS and agitate vigorously for 30 minutes.
3. Centrifuge for 10 minutes at 3000 g.
4. Dilute the clear supernatant 1:6 in Sample dilution buffer and test this sample in the ELISA.
5. Sample dilution factor: **F=24**

Liver

1. Mill and homogenize sample with an appropriate device (mixer, ultra-turrax).
2. Mix 1 g sample with 4 ml 0.01 M PBS and agitate vigorously for 30 minutes.
3. Centrifuge for 10 minutes at 3000 g.
4. Dilute the clear supernatant 1:8 in Sample dilution buffer and test this sample in the ELISA.
5. Sample dilution factor: **F=32**

Milk

1. Refrigerate to 2-8°C and centrifuge at 3000 g for 10 minutes.
2. Remove or penetrate the upper fat layer, dilute milk 1:8 in Sample

dilution buffer and test this sample in the ELISA.

3. Sample dilution factor: **F=8**

Whole Egg (raw)

1. Homogenize sample with an appropriate device (mixer, vortex, or ultra-turrax).
2. Add 250 µl of Carrez I solution to 5 ml egg sample, mix well and add 250 µl of Carrez II solution afterwards.
3. Mix sample and centrifuge at 3000 g for 10 minutes.
4. Dilute the supernatant 1:15 in Sample dilution buffer and test this sample in the ELISA.
5. Sample dilution factor: **F=16.5**

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 10X wash buffer into distilled water to yield 1X wash buffer. The 1X Wash buffer is stable for 1 month at 2-8 °C. Mix well before use.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Pipet **100 µl** of **standards or prepared samples** in duplicate into the appropriate wells of the microtiter plate. Immediately add **50 µl** of **anti-streptomycin antibody** into each well.
3. Cover the microtiter plate with a plastic foil and **incubate for 30 minutes at room temperature.**
4. Without preceding washing add **50 µl** of **HRP-Streptomycin Conjugate** into each well.
5. Cover the microtiter plate with a plastic foil and **incubate additional 15 minutes at RT.**
6. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes**. Wash by filling each well with **1X wash buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
7. Add **100 µl** of **TMB mixture** to each well. **Incubate for 15 minutes at room temperature** in dark.
8. Add **100 µl** of **Stop Solution** to each well.
9. Read the OD with a microplate reader at **450 nm** immediately. (Optional:

read reference wavelength at 620 nm). It is recommended read the absorbance within 30 min after adding STOP solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

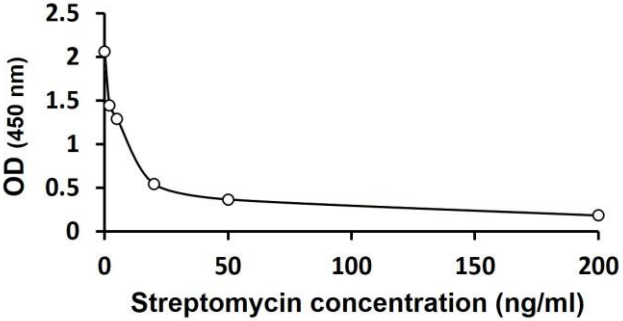
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. The diluted samples must be further converted by the **appropriate dilution factor** according to the sample preparation procedure as described above.

Note: Due to matrix effects some negative samples may show a certain blank value. In validation experiments this was determined to be around 1-2 ng/mL. This value has to be considered as the limit of detection of the method.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The sensitivity of the Streptomycin ELISA is 1 ng/mL (based on the standard curve).

Specificity

Cross-reactivity	Relative to streptomycin (=100%)
Dihydrostreptomycin	70%
Gentamycin	< 0.001%
Neomycin	< 0.001%

Intra-assay

The CV value of intra-assay precision was 6%.

Recovery

Honey	100%
Shrimps	70%
Meat	90%
Liver	95%
Milk	120%
Whole egg	85%