# Test Instruction

Streptomycin

96 Tests

Enzyme Immunoassay
for the Quantitative
Determination of
Streptomycin in Food

Cat.-No.: STR-E02

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| | |
|---|---|
| Sensitivity | 1 ng/mL |
| Recovery (spiked samples) | 70-120% |
| Incubation Time | 60 min |

## General Information

Streptomycin consists of three components, which are linked together by glycoside bonds, and it belongs 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 tubercle 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 veterinary area. After the treatment of mastitis in breeding 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 values were defined in the European Union. The German critical value for streptomycin in honey according to the RHmV regulation is at the moment 20 µg/kg.

## Principle of the Test

The **Gold Standard Diagnostics Streptomycin** quantitative test is based on the principle of the enzyme-linked immunosorbent assay. 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 colour. The colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm. The concentration of streptomycin is indirectly proportional to the colour intensity of the test sample.

## Precautions

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results:

1. Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).

2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.

3. Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.

4. Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.

5. Use a separate disposable tip for each specimen to prevent cross-contamination.

6. All specimens and standards should be run at the same time, so that all conditions of testing are the same.

7. Do not mix components from different batches.

8. Do not use reagents after expiration date.

9. Check both precision and accuracy of the laboratory equipment used during the procedure (micropipets, ELISA reader etc.).

## Health and safety instructions

1. Do not smoke or eat or drink or pipet by mouth in the laboratory.

2. Wear disposable gloves whenever handling patient specimens.

3. Avoid contact of substrate and stop solution with skin and mucosa (possible irritation, burn or toxicity hazard). In case of contact, rinse the affected zone with plenty of water.

4. Handling and disposal of chemical products must be done according to good laboratory practices (GLP).

## Reagents

The kit contains reagents for 96 determinations. They have to be stored at 2-8°C. Expiry data are found on the labels of the bottles and the outer package.

1. Microtiter plate consisting of 12 strips with 8 breakable wells each, coated with anti-mouse antibody.

2. Streptomycin Standards (0, 2, 5, 20, 50, 200 ng/mL): 6 vials with 1 mL each, dyed red, ready-to-use.

3. Anti-Streptomycin Antibody (mouse): 6 mL, dyed red, ready-to-use.

4. Conjugate (Streptomycin-Peroxidase): 6 mL, dyed red, ready-to-use.

5. Substrate Solution (TMB): 15 mL, prestained red, ready-to-use.

6. Stop Solution (0.5 M $H_2SO_4$): 15 mL, ready-to-use.

7. Sample Diluent (PBS): 2 x 60 mL, dyed red, ready-to-use.

8. Washing Solution (PBS + Tween 20): 60 mL as 10x concentrate. Dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.

9. Two plastic foils to cover the strips during the incubation.

10. Plastic bag to store unused microtiter strips.

11. Instruction Manual.

## Additional Instrumentation and Reagents (not provided)

### *Instrumentation*

- 50, 100, 500 and 1000 µL-micropipets
- ELISA reader (450 nm)
- Centrifuge
- Ultra-Turrax, mixer, vortex

### *Reagents*

- Double distilled water
- 0.01 M PBS (8.77 g/L NaCl, 0.7 g/L $NaH_2PO_4x2H_2O$, 2.9 g/L $Na_2HPO_4x2H_2O$, pH 7.3)
- Extraction buffer (2.0 g heptanesulfonic acid sodium salt, 1.9 g $Na_3PO_4x12H_2O$ ad 200 mL double distilled water, adjust pH 2.0 with o-phosphoric acid)
- Methanol (100%)
- Potassiumhexacyanoferrate(II)-3-hydrate (150 g/L; Carrez I)
- Zincsulfate-7-hydrate (300 g/L; Carrez II)

## Sample Preparation

### *Honey (Screening Method)*

- Dissolve 2 g honey sample in 10 mL double distilled water.
- Further dilute this extract 1:4 with sample diluent.
- Sample dilution factor: F=20

### *Honey (Sensitive Method; C18 SPE)*

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

### *Shrimps*

- Mill and homogenize sample with an appropriate device (mixer, ultra-turrax).
- Mix 1 g sample with 4 mL 0.01 M PBS and agitate vigorously for 30 minutes.
- Centrifuge for 10 minutes at 3000 g.
- Dilute the clear supernatant 1:4 in sample diluent and test this sample in the ELISA.
- Sample dilution factor: F=16

### *Meat*

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

### *Liver*

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

### *Milk*

- Refrigerate to 2-8°C and centrifuge at 3000 g for 10 minutes.
- Remove or penetrate the upper fat layer, dilute milk 1:8 in sample diluent and test this sample in the ELISA.
- Sample dilution factor: F=8

### *Whole Egg (raw)*

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

## Procedure

1. Prepare samples as described above.

2. Pipet 100 µL standards or prepared samples in duplicate into the appropriate wells of the microtiter plate. Immediately add 50 µL 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 streptomycin-peroxidase conjugate into each well.

5. Cover the microtiter plate with a plastic foil and incubate additional 15 minutes at room temperature.

6. Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Pipet 300 µL of diluted washing solution into each well. After the third repetition empty the wells again and remove residual liquid by striking the plate against a paper towel. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbencies.

7. Pipet 100 µL of substrate solution into each well.

8. Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 15 minutes at room temperature.

9. Stop enzyme reaction by adding 100 µL of stop solution (0.5 M $H_2SO_4$) into each well. The blue colour will turn yellow upon addition.

10. After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The colour is stable for 30 minutes.

## Calculation of results

1. Calculate the average optical density (OD 450 nm) for each set of reference standards or samples.

2. Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in ng/mL on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis.

3. Using the mean optical density value for each sample, determine the corresponding concentration of streptomycin in ng/mL from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.

4. The diluted samples must be further converted by the appropriate **sample dilution factor**. The factors are listed for each sample matrix in the *sample preparation* section.

**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.**

## Typical Standard Values

The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 0 ng/mL standard. These values are only an example and should not be used instead of the standard curve which has to be measured in every new test.

| Streptomycin (ng/mL) | (% binding of 0 ng/mL) |
|---|---|
| 0 | 100 |
| 2 | 73 |
| 5 | 53 |
| 20 | 33 |
| 50 | 14 |
| 200 | 7 |

## Performance

### *Sensitivity*

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

### *Recovery*

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

### *Intra-assay Precision*

The intra-assay variation of the streptomycin test was determined to 6%.

### *Cross-reactivity relative to streptomycin (=100%)*

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|---|---|
| Dihydrostreptomycin | 70% |
| Gentamycin | < 0.001% |
| Neomycin | < 0.001% |

## References


1. Suhren G, Knappstein K; *Analyst*. 1998 Dec;123(12): 2797-801:
   Detection of incurred dihydrostreptomycin residues in milk by liquid chromatography and preliminary confirmation methods.
2. Edder P, Cominoli A, Corvi C; *J Chromatogr A*. 1999 Jan 15; 830(2):345-51:
   Determination of streptomycin residues in food by solid-phase extraction and liquid chromatography with post-column derivatization and fluorometric detection.
3. Haasnoot W, Stouten P, Cazemier G, Lommen A, Nouws JF, Keukens HJ; *Analyst*. 124(3): 301 (1999):
   Immunochemical detection of aminoglycosides in milk and kidney.