Antibiotics ELISA kits available from ADI:

http://4adi.com/commerce/catalog/spcategory.jsp?category_id=2739

DE-100010	Clenbuterol ELISA kit, 96 tests (For Urine, Serum, Feed, Meat, Liver)
DE-100020	Ractopamine ELISA kit, (For Liver, Urine, Feed), 96 tests
DE-100030	Salbutamal ELISA kit, For Urine, Tissue, Feed, Animal Tissue, Aquatic, Honey, Intestine,, 96 tests
DE-100040	Chloramphenicol ELISA kit, 96 tests (For Animal Tissue, Aquatic, Honey, Intestine, Urine, Egg, Milk, Serum)
DE-100050	Florfenicol ELISA kit (For Animal Tissue, Aquatic, Honey), 96 tests
DE-100060	Nitrofuran (AMOZ) ELISA kit (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100070	Nitrofuran (AHD) ELISA kit, (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100075	Nitrofuran (SEM) ELISA kit (Honey, Fish, Shrimp, Chicken/Liver, Fish/Shrimp), 96 tests
DE-100080	Nitrofuran (AOZ) ELISA kit (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100090	Sulfonamides Residues (SAs) ELISA kit, (For Chicken/Liver, Pork/Liver, Honey/Egg, Serum/Urine, Milk), 96 tests
DE-100100	Sulfamethazine (SM2) ELISA kit, 96 tests (For Chicken/Liver, Pork/Liver, Honey/Egg, Serum/Urine, Milk)
DE-100110	Sulfamethoxydiazine (SMD) ELISA kit, (For Chicken/Liver, Pork/Liver, Honey/Egg, Serum/Urine), 96 tests
DE-100120	Quinolones (QNS) ELISA kit (For Pork/Liver, Chicken/Liver, Shrimp, Fish, Serum, Honey), 96 tests
DE-100130	Enrofloxacin ELISA kit (For Pork/Liver, Chicken/Liver, Shrimp, Fish, Serum, Honey), 96 tests
DE-100140	Ampicillin ELISA kit, (For Pork/Liver, Chicken, Duck, Shrimp, Fish, Honey, Milk), 96 tests
DE-100150	Benzyl Penicillin ELISA kit, (For Pork/Liver, Chicken, Duck, Shrimp, Fish, Honey, Milk), 96 tests
DE-100160	Tylosin ELISA kit (For Meat, Liver, Honey, Egg), 96 tests
DE-100170	Trenbolone ELISA kit (For Animal Tissue, Aquatic, Urine), 96 tests
DE-100180	Diazepam ELISA kit (For Tissue, Urine, Feed), 96 tests
DE-100190	Diethylstilbestrol (DES) ELISA kit (Fish, Shrimp, Liver, Meat, Feed, Urine), 96 tests
DE-100200	Gentamicin ELISA kit (Chicken/Liver), 96 tests
DE-100210	Streptomycin ELISA kit, 96 tests (Chicken/Liver, Honey, Milk)
DE-100230	Olaquindox ELISA kit (Tissue) 96 tests
DE-100240	Sulfaquin-oxaline ELISA kit, (For Pork/Liver, Honey/Egg, Serum/Urine, Milk), 96 tests

See Details at the web site or Contact ADI

Instruction Manual No. ***M-DE-100210***

Streptomycin ELISA KIT

Cat. #DE-100210

For Qualitative and Quantitative Determination of Streptomycin in milk, chicken, and honey.

For in vitro research use only (RUO), not for therapeutic or diagnostic use.

ALPHA DIAGNOSTIC INTERNATIONAL

India Contact:

Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034
Ph: +91-11-42208000, 42208111, 42208222

Mobile: +91-9810521400
Fax: +91-11-42208444
Email: customerservice@atzlabs.com
Web: www.atzlabs.com

LIFE TECH Life Technologies™
Your Molecular & Cell Technology Partner

DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

Streptomycin ELISA KIT Cat. #DE-100210

Kit Components, 96 tests	t #
Micro-well coated strip plate (12 strips with 8 removable wells each) # DE100211	1 plate
6x standard solution (1 ml each): #DE100212E-F 0.0 ppb, 0.1 ppb, 0.4 ppb, 1.6 ppb, 6.4 ppb, 25.6 ppb	6 vials
Enzyme conjugate (12 mL) # DE100213; red cap	1 bottle
Antibody solution (7 mL) # 100214; blue cap	1 bottle
Sample buffer (10X) 50 ml #100215; transparent cap	1 bottle
Washing buffer (20X), 40 ml, 100210WB; white cap	1 bottle
Substrate A solution (7 mL) 100210SA; white cap	1 bottle
Substrate B solution (7 mL) 100210SB; black cap	1 bottle
Stop solution (7 mL) 100210SS; yellow cap	1 bottle
Instruction Manual	M-DE100210

Intended use

Alpha Diagnostic Intl's Streptomycin ELISA kit is a highly sensitive competitive type ELISA for the measurement of Streptomycin in chicken liver, honey and milk. For in vitro research use only (RUO), not for therapeutic or diagnostic use.

Introduction

Aminoglycoside is a molecule formed by a sugar and an amino group. Many aminoglycoside has a role as an antibiotic. Some examples of aminoglycoside are amikacin, arbekacin, gentamicin, kanamycin, neomycin, netilmicin, paromomycin, ryhodostreptomycin, tobramycin, apramycin and streptomycin. Streptomycin was the first antibiotic drug to be used to treat tuberculosis. Its molecular formula is C12H39N7O12, its molecular weight is 581.574 g/mol and it is derived from the actinobacterium Streptomyces griseus, a soil bacteria. Streptomycin was first isolated by Albert Schatz in October 19, 1943. He was a graduate student of Rutgers University. Streptomycin mechanism works by inhibiting protein synthesis and killing the microbes. Streptomycin binds to the 16S rRNA of the bacterial ribosome and interferes with the binding of formyl-methionyl-tRNA to the 30S subunit, blocking the start of protein synthesis, which eventually leads to cell death. Since human and bacteria ribosomes are structured differently it makes the antibiotic be selective for bacteria. When streptomycin is given in low concentration it inhibits only the growth of the bacteria by stimulating prokaryotic ribosomes to misread mRNA. Streptomycin should be injected into the muscle and not taken orally. One side effect of this medicine is that it can lead to temporary hearing loss, ototoxicity. Other side effects include nausea, vomiting, rash, fever, urticaria, eosinophilia, angioneurotic edema, and paresthesia of face. Taking streptomycin during pregnancy can be very harmful to the fetus since streptomycin can easily pass trough the placental.

In human, besides the use to treat tuberculosis, streptomycin has also been used to treat plague and infective endocarditis. In animals such as horse, cattle, and sheep, streptomycin is the first antibiotic option to use against gram-negative bacteria. Besides the use in medicine, it will work against the growth of bacteria, fungi, and algae, as a pesticide. It is used to control bacterial and fungal diseases of some fruits, vegetables, seed and crops.

Qualitative determination

The concentration range (ng/mL) can be obtained from comparing the average OD value of the testing sample with that of the standard solution. Assuming that the OD value of the sample Ⅰ is 0.3, and that of the sample Ⅱ is 1.0, the OD value of standard solutions is: 2.243 for 0 ppb, 1.816 for 0.5 ppb, 1.415 for 1.5 ppb, 0.74 for 4.5 ppb, 0.313 for 13.5 ppb, 0.155 for 40.5 ppb, accordingly the concentration range of the sample Ⅰ is 13.5 to 40.5 ppb, and that of the sample Ⅱ is 1.5 to 4.5 ppb.

Quantitative determination

The mean values of the absorbance values is obtained for the average OD value (B) of the sample and the standard solution divided by the OD value (B0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is,

$$\text{Percentage of absorbance value} = \frac{B}{B0} \times 100\%$$

B—the average OD value of the sample or the standard solution
B0—the average OD value of the 0 ng/mL standard solution

Draw the standard curve with the absorption percentages of the standard solution and the semilogarithm values of the Streptomycin standard solution (ng/mL) as Y- and X-axis, respectively. Read the corresponding concentration of the sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the corresponding dilution fold, finally obtaining the Streptomycin concentration in the sample. Using the professional analyzing software of this kit will be more convenient for the accurate and rapid analysis of a large amount of samples. (Please contact us for this software).

Technical specifications

Sensitivity: 0.1 ppb

Detection limit

Chicken.....1 ppb
Chicken, liver, milk.....4 ppb
Honey Royal jelly.....2 ppb

Recovery rate

Milk.....85±22%
Chicken, tissue.....80±22%
Honey, Royal jelly.....75±22%

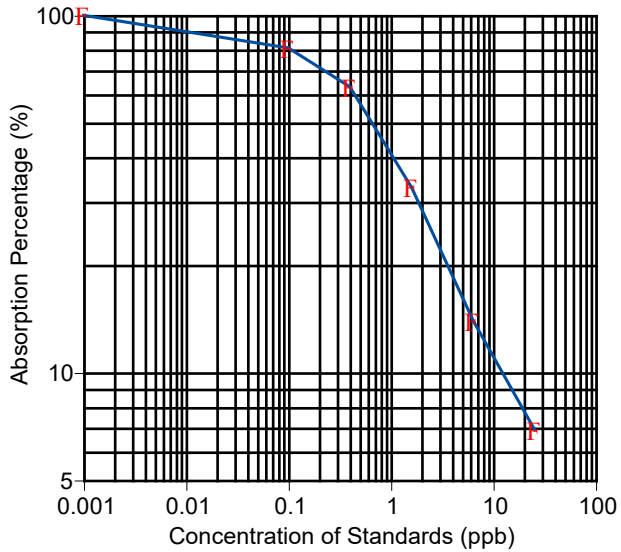
Cross-reactivity

Streptomycin.....100%
Dihydrostreptomycin.....108%
Kalamycin.....<0.1%
Gentamycin.....<0.1%

Work Sheet of Typical Assay-Streptomycin

Wells	Stds/samples		Mean A_{450} nm	Absorption Percentage
A1, A2	**Standard A**	0.0 ppb	2.243	100%
B1, B2	**Standard B**	0.1 ppb	1.816	86.96%
C1, C2	**Standard C**	0.4 ppb	1.415	63.09%
D1, D2	**Standard D**	1.6 ppb	0.74	32.99%
E1, E2	**Standard E**	6.4 ppb	0.313	13.95%
F1, F2	**Standard F**	25.6 ppb	0.155	6.91%

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical assay Standard Curve (do not use this for calculating sample values)

CALCULATION OF RESULTS

There are two methods to judge the results: the first one is the rough judgment, while the second is the quantitative determination. Note that the OD value of the sample has a negative correlation with the Streptomycin concentration.

PRINCIPLE OF THE TEST

This test kit is based on the competitive enzyme immunoassay for the detection of Streptomycin in the sample. The coupling antigen is pre-coated on the micro-well stripes. The Streptomycin in the sample and the coupling antigens pre-coated on the micro-well stripes compete for anti-Streptomycin antibodies. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the testing sample has a negative correlation with the Streptomycin concentration in the sample. This value is compared to the standard curve and the Streptomycin concentration is subsequently obtained.

MATERIALS AND EQUIPMENT REQUIRED

Equipments: microplate reader, printer, vortex, centrifuge, homogenizer, measuring pipettes and balance (a sensibility reciprocal of 0.01 g)
Micropipettors: single-channel 20 to 200 µL and 200 to 1000 µL, and multi-channel 250 µL.
Reagents: Methanol, $Na_2HPO_4 \cdot 12H_2O$, $NaH_2PO_4 \cdot 2H_2O$, NaCl, Heptanesulfonate sodium.

PRECAUTIONS AND SAFETY INSTRUCTIONS

The Streptomycin Kit is for research use only.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI web site. TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).
http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

SAMPLE PRE-TREATMENT

Instructions

The following points must be dealt with before the pre-treatment of any kind of sample:

1. Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents.
2. Before the experiment, each experimental equipment must be clean and should be re-cleaned if necessary, in order to avoid the contamination that interferes with the experimental results.

Solution required but not provided

1. **0.5% Trichloroacetic acid solution**: dissolve 0.5g Trichloroacetic acid in the deionized water to 100 mL.
2. **2.3% Trichloroacetic acid solution**: dissolve 3g Trichloroacetic acid in the deionized water to 100 mL.
3. **0.1M NaOH**: dissolve 0.4 g NaOH in the deionized water to 100 mL.

Samples preparation

a) Milk

1 Take 1 mL milk sample, diluted at 1:39 (1950 µL diluted redissolving solution+ 50 µL milk). Mix for 30 seconds
2 Take **50 µL** for analysis. **Fold of dilution of sample :40**

b) chicken meat

1 Take 2±0.05 g homogenized sample(remove fat), add 4 mL 3% Trichloroacetic acid. Mix for 2 min.
2 Centrifuge at above 4000 r/min at room temperature for 10 min.
3 Transfer 100 µL supernatant into a new vessel, then add 100µl 0.1M NaOH and 300 µL the diluted redissolving solution, mix properly for 30 seconds.
4 Take **50 µL** for analysis. **Fold of dilution of sample: 10**

c) chicken liver

1 Take 2±0.05 g homogenized sample(remove fat), add 6mL 0.5% Trichloroacetic acid and 2ml . CH3CN, mix for 5 min.
2 Centrifuge at above 4000 r/min at room temperature for 10 min.
3 Transfer 2ml supernatant into a new vessel, then add 2ml N-hexane, mix evenly, be static for 3 min, take 0.5ml down-layer bright solution, centrifuge at above 4000 r/min at room temperature for 5min.
4 Take 50µl down-layer bright solution (If there is layering, remove the up-layer, take the down-layer bright solution), add 450µl the diluted redissolving solution, mix for 30s.
5 Take **50 µL** for analysis. **Fold of dilution of sample: 40**

d) Honey, Royal jelly

1 Weigh 1±0.05 g honey sample, add 2 mL of de-ionized water,shake properly until dissolved completely.
2 Centrifuge at above 4000 r/min at room temperature (20-25 ℃) for 10 min, until liquid is clear.
3 Take 100 µL supernatant, add 400 µL of the diluted redissolving solution, mix evenly. For 30 seconds
4 Take **50 µL** for further analysis. ***Fold of dilution of sample :10***

STORAGE AND STABILITY

Storage: store at 2 to 8 ℃, not frozen.
Expiration date: 12 months; date of production is on box.

Reagent Preparation

Wash buffer: Dilute 1:20 with water (40 mL 20X mixed with 760 ml water. Or Prepare as needed. Store 1X wash buffer at room temp for the duration of the use and store at 4oC until the kit expiration.

Sample buffer: Dilute 1:10 with water (50 mL 10X mixed with 450 ml water. Or Prepare as needed. Store 1X wash buffer at room temp for the duration of the use and store at 4oC until the kit expiration.

Assay Procedure

Bring test kit to the room temperature (20-25 ℃) for at least 30 min, note that each reagent must be shaken evenly before use; put the required micro-well strips into plate frames. Resealed the unused microplate, stored at 2-8 ℃, not frozen.

1. Add **50 µL** of **the standard and sample in** duplicate wells.
2. Add **50 µL of the antibody solution** to each well, mix gently manually for 5-10 seconds. Cover the microplate and incubate at **37°C for 30 min**.
3. Aspirate the plates and tap dry on absorbent paper. Wash the plate 5 times with 300 ul wash buffer (1X).
4. Add **100 µL enzyme conjugate** to each well. Mix gently manually for 5-10 second, cover the plate and incubate at **37 °C for 30 min**. Aspirate the plates and tap dry on absorbent paper**. Wash 5 times** same as in step 3.
5. Add **50 µL** of the **substrate A** solution followed by **50 µL of the B solution** into each well. (alternatively it is possible to mix Soln A and B in 1:1 ratio in a clean tube, mix, and add 100 ul of the mix in a single step). Mix gently manually for 5-10 seconds. Cover the plate and incubate at **37 °C for 15-20 min** in the dark (blue color develops in standards and samples).
6. Add **50 µL** of the **stop solution** into each well. Mix gently manually for 5-10 seconds (blue color turns yellow). Read the plate at 450 nm to determine the OD value of every well. (Recommend to read the OD value at the dual-wavelength 450/630nm) within 5-10 min.

NOTES:

1. Return all reagents to 2-8 °C immediately after use.
2. The reproducibility of the ELISA analysis, to a large degree, depends on the consistency of plate washing. The correct operation of plate washing is the key point in the procedures of ELISA.
3. For the incubation at constant temperatures, all the samples and reagents must avoid light exposure, and each microplate should be sealed by the cover membrane.
4. Dryness of the microplate in the washing process will be accompanied by the situations including the non-linear standard curves and the undesirable reproducibility; So continue to next step immediately after washing.
5. Mix evenly, otherwise there will be the undesirable reproducibility.
6. The stop solution contains sulfuric acid solution, avoid contacting with the skin.
7. Do not use the kit exceeding its expiry date. The use of diluted or adulterated reagents from the kits will lead to the changes in the sensitivity and the detecting OD values. Do not exchange the reagents from the kits of different lot numbers to use.
8. Put the unused microplate into an auto-sealing bag to re-seal it. The standard substance and the colorless color former is light sensitive, and thus they cannot be directly exposed to the light.
9. Discard the coloration solution with any color that indicates the degeneration of this solution. The detecting value of standard solution 1（0 ppb）of less than 0.5 indicates its degeneration.
10. Substrate time is about 20 min, if the color is light , prolong the time of coloration but don't exceed 30 min.
11. The optimum reaction temperature is 37 °C, and too high or low temperatures will result in the changes in the detecting sensitivity and OD values.