Streptomycin ELISA Kit (OKAO00125)

Lot# KH0607

Instructions for Use

For the quantitative detection of Streptomycin (SDM) concentration in tissue, honey, royal jelly, milk, milk powder, egg.

This product is intended for research use only.

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1. Background

Principle

Aviva Systems Biology Streptomycin ELISA Kit (OKAO00125) is based on standard competitive inhibition enzyme-linked immuno-sorbent assay technology. Streptomycin has been pre-coated onto a 96-wellplate (12 x 8 Well Strips). Standards or test samples are added to the wells along with a rabbit anti-Streptomycin antibody and an anti-rabbit / HRP Conjugated antibody, then incubated. Wells are washed and detection substrates are added and incubated. An enzymatic reaction is produced through the addition of TMB which is catalyzed by HRP generating a blue color product that changes yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm which is quantitatively proportional to the amount of detector antibody bound in the well and inversely proportional to the amount of Streptomycin in the sample.

Background

Streptomycin is an antibiotic (antimycobacterial) drug, the first of a class of drugs called aminoglycosides to be discovered, and it was the first effective treatment for tuberculosis. It is derived from the actinobacterium Streptomyces griseus. Streptomycin is a bactericidal antibiotic. Adverse effects of this medicine are ototoxicity, nephrotoxicity, fetal auditory toxicity, and neuromuscular paralysis. It is on the World Health Organization's List of Essential Medicines, a list of the most important medication needed in a basic health system.

General Specifications

General Specifications	
Detection Range	0.1 – 8.1 ppb
Limit of Detection	0.1 ppb
Specificity	Streptomycin CAS#: 57-92-1 PubChem#: 19649 Alias: SM; Streptomycin; Streptomycin A; Agrimycin; Strepcen; Agrept; Gerox

2. Storage and Stability

• Upon receipt store kit at 4°C. Do not use past expiration date.
• The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 5% within the expiration date under appropriate storage condition.

3. Kit Components

•The following reagents are the provided contents of the kit.

Description	Quantity	Storage Conditions
Streptomycin Microplate	96 Wells (12 x 8 Well strips)	4°C Do not use past expiration date
Streptomycin Standards	6 x 1 mL	
Streptomycin Detector Antibody	1 x 6 mL	
HRP Conjugate	1 x 11 mL	
Detection Reagent A	1 x 6 mL	
Detection Reagent B	1 x 6 mL	
5X Sample Prep Buffer	1 x 50 mL	
20X Wash Buffer	1 x 20 mL	
Stop Solution	1 x 6 mL	

4. Required Materials Not Supplied

• Microplate reader capable of reading absorbance at 450 nm.
• Automated plate washer (optional).
• Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
• Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
• New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
• Absorbent paper or paper toweling.
• Distilled or deionized ultrapure water.
• Heater water bath
• Centrifuge
• Reagents: NaOH, n-Hexane, $Na_2HPO_4 \cdot 12H_2O$, $NaH_2PO_4 \cdot 2H_2O$, H_3PO_4

5. Precautions

• Read instructions fully prior to beginning use of the assay kit.

• Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.

• Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.

• For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).

• Kit cannot be used beyond the expiration date on the label.

6. Technical Application Tips

• Do not mix or substitute components from other kits.

• To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.

• Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.

• Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.

• Replicate wells are recommended for standards and samples.

• Cover microplate while incubating to prevent evaporation.

• Do not allow the microplate wells dry at any point during the assay procedure.

• Do not reuse tips or tube to prevent cross contamination.

• Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.

• Completely remove of all liquids when washing to prevent cross contamination.

• Prepare reagents immediately prior to use and do not store, with the exception of the top standard.

• Equilibrate all materials to ambient room temperature prior to use (standards exception).

• For optimal results for inter- and intra-assay consistency, equilibrate all materials to room temperature prior to performing assay (standards exception) and perform all incubations at 37°C.

• Pipetting less than 1 µL is not recommended for optimal assay accuracy.

• Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.

• Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.

• Samples containing precipitates, fibrin strands or bilirubin, or are hemolytic or lipemic might cause inaccurate results due to interfering factors.

• TMB Substrate is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

• To minimize influences on the assay performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.

7. Reagent Preparation

• Equilibrate all materials to room temperature prior to use and use prepare immediately prior to use.

• The following reagents are provided at ready to use concentrations and require no preparation:

- Streptomycin Standards
- Streptomycin Detector Antibody
- HRP Conjugate
- Detection Reagent A
- Detection Reagent B

7.1 Standards

Standards are provided at ready to use concentrations. The concentrations are as follows:

Tube	Standard 5	Standard 4	Standard 3	Standard 2	Standard 1	Standard 0
ppb	8.1	2.7	0.9	0.3	0.1	0

7.2 1X Wash Buffer

7.2.1 Add the 20 mL of **20X Wash Buffer** to 380 mL of ultrapure water.

7.2.2 Mix gently and thoroughly. Store at 4°C for one week.

7.3 1X Sample Prep Buffer

7.3.1 Add the 50 mL of **5X Sample Prep Buffer** to 200 mL of ultrapure water.

7.3.2 Mix gently and thoroughly. Store at 4°C for one week.

7.4 0.4 M H_3PO_4 (for honey sample)

7.4.1 Dissolve 1 mL H_3PO_4 in deionized water to 360 mL.

7.4.2 Mix gently and thoroughly. Store at 4°C for one week.

7.5 1 M NaOH solution (for honey sample)

7.5.1 Dissolve 4 g NaOH in the deionized water to 100 mL.

7.5.2 Mix gently and thoroughly. Store at 4°C for one week.

7.6 1% Acetic Acid Solution (for egg sample)

7.6.1 Dissolve 1 mL Acetic acid in deionized water to 100 mL.

7.6.2 Mix gently and thoroughly. Store at 4°C for one week.

7.7 70% Methanol Solution (for egg sample)

7.7.1 Dissolve 700 mL methanol in deionized water to 1000 mL.

7.7.2 Mix gently and thoroughly. Store at 4°C for one week.

7.8 0.05 M PB Buffer

7.8.1 Dissolve 12.9 g $Na_2HPO_4 \cdot 12H_2O$, 2.17 g $NaH_2PO_4 \cdot 2H_2O$ in L of deionized water.

7.8.2 Mix gently and thoroughly. Store at 4°C for one week.

7.9 Microplate Preparation

- Micro-plates are provided ready to use and do not require rinsing or blocking.
- Unused well strips should be returned to the original packaging, sealed and stored at 4°C.
- Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

8. Sample Preparation

8.1 Sample Preparation and Storage

- Mix diluted samples gently and thoroughly.
- Pipetting less than 2 µL is not recommended for optimal assay accuracy.
- Untreated samples should be stored at -20°C.
- Prepared samples can be stable for 1 week at 4°C.

8.1.1 **Tissue (Dilution 1:40)**

8.1.1.1 Homogenize the sample.

8.1.1.2 Weigh 2.0 ± 0.05 g of the homogenized sample, add 8 mL **0.05 M PB** buffer.

8.1.1.3 Shake for vigorously for 5 minutes, then keep in 56°C water bath for 30 minutes.

8.1.1.4 Centrifuge at above 4,000 rpm at room temperature (20-25 °C) for 10 minutes.

8.1.1.5 Transfer 1 mL of supernatant into a new centrifugal tube.

8.1.1.6 Add 1 mL **N-hexane** to dry residue, shake for 2 minutes.

8.1.1.7 Centrifuge at 4,000 rpm at room temperature for 5 minutes, remove the upper layer.

8.1.1.8 Add 450 µL of **1X Sample Prep Buffer** and mix evenly for 30 seconds.

8.1.1.9 Take 50 µL for further analysis.

8.1.2 **Honey, royal jelly (Dilution 1:20)**

8.1.2.1 Weight 2.0 ± 0.05 g honey sample, then add 4 mL **0.4 M H_3PO_4.** Shake properly for 10 minutes.

8.1.2.2 Centrifuge greater than 4,000 rpm at room temperature (20-25°C) for 5 minutes (honey could be operated at step 2 without centrifuging).

8.1.2.3 Add 450 µL of **1 M NaOH**, adjust pH to 7-9 (for royal jelly, transfer supernatant to new centrifugal tube, then adjust pH to 7-9).

8.1.2.4 Centrifuge greater than 4,000 rpm at room temperature (20-25°C) for 5 minutes, until liquid is clear.

8.1.2.5 Take 50 µL of the supernatant

8.1.2.6 Add **450 µL of 1X Sample Prep Buffer** and mix for 30 seconds.

8.1.2.7 Take 50 µL for further analysis.

8.1.3 **Milk, Milk Powder (Diluted 1:50)**

8.1.3.1 Weigh 2 ± 0.05 g milk powder (or 2 mL milk).

8.1.3.2 Add 8 mL **0.05 M PB buffer**, mix for 5 minutes.

8.1.3.3 Keep in 56°C water bath for 30 minutes.

8.1.3.4 Centrifuge at above 4,000 rpm at room temperature (20-25 °C) for 10 minutes.

8.1.3.5 Remove upper layer fat, take 50 µL into a new centrifugal tube.
8.1.3.6 Add 450 µL of **1X Sample Prep Buffer**, mix for 30 seconds.
8.1.3.7 Take 50 µL for further analysis.

8.1.4 **Egg (Diluted 1:100)**
8.1.4.1 Weigh 1 ± 0.05 g homogenized sample into 50 mL centrifugal tube.
8.1.4.2 Add 2 mL of **Acetic Acid solution**, shake for 2 min.
8.1.4.3 Add 7 mL of 70% **Methanol solution**, shake for 2 min.
8.1.4.4 Centrifuge at above 4,000 rpm at room temperature (20-25 °C) for 10 minutes.
8.1.4.5 Transfer 0.1 mL supernatant (must be clear) into centrifuge tube.
8.1.4.6 Add 900 µL of **1X Sample Prep Buffer**, mix for 30 seconds.
8.1.4.7 Take 50 µL for further analysis.

9. Assay Procedure

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

• Temperature regulation for all procedures and incubations to 25°C is recommended for optimal reproducibility.

9.1 Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.

9.2 Add 50 µL of standards or samples into wells of the **Streptomycin Microplate**. At least two replicates of each standard, sample or blank is recommended.

9.3 Immediately add 50 µL of the **Streptomycin Detector Antibody** and 50 µL of the **HRP Conjugate** to each well.

9.4 Cover the plate with the plate sealer, gently mix and incubate for 45 minutes.

9.5 Remove the plate lid and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

9.6 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

9.7 Wash plate 5 times with **1X Wash Buffer** as follows:

9.7.1 Add 250 µL of **1X Wash Buffer** to each assay well.
9.7.2 Incubate for 30 seconds.
9.7.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.
9.7.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
9.7.5 Repeat steps 9.7.1 through 9.7.4 **four** more times.

9.8 Add 50 µL of prepared **Detection Reagent A** and 50 µL of **Detection Reagent B** to each well.

9.9 Gently mix the plate and incubate for 15 minutes **in the dark**.

9.10 Add 50 µL of **Stop Solution** to each well and mix gently. Well color should change to yellow immediately. Add the **Stop Solution** in the same well order as done for the **Detection Reagents**.

9.11 Read the O.D. absorbance at 450 nm with a standard microplate reader within 5 minutes of stopping the reaction in step 9.10. If wavelength correction is available, set to 540 nm or 570 nm.

10. Calculation of Results

For analysis of the assay results, first derive the **Percent Relative Absorbance** for each sample or standard by calculating the mean absorbance between replicate wells. This value is then divided by the mean replicate Blank (0 ppb) absorbance, then multiplying by 100%:

$$\text{Percent Relative Absorbance (Sample or Standard)} = \frac{\text{Mean Absorbance (Sample or Standard)}}{\text{Mean Blank Absorbance}} \times 100\%$$

A standard curve is generated by plotting the **Percent Relative Absorbance** of each standard serial dilution point vs. the respective standard concentration. The Streptomycin concentration contained in the samples can be interpolated by using linear regression of each sample **Percent Relative Absorbance** against the standard curve. This is best achieved using curve fitting software.

Note: If wavelength correction readings were available, subtract the readings at 540 nm or 570 nm from the readings at 450 nm. This may provide greater reading accuracy.

Note: If the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

11. Typical Expected Data

11.1 Limit of Detection

Derived by linear regression of OD450 of the Mean Blank + 2xSD

Sample	Mean Recovery
Tissue	4 ppb
Honey, Royal jelly	2 ppb
Milk, Milk powder	5 ppb
Egg	10 ppb

11.2 Reproducibility

Three samples of known concentrations were measured in 20 replicates within an assay plate to asses intra-assay reproducibility:

Mean Intra-Assay Precision - ≤8% (n = 3 x 20)

Three samples of known concentrations were measured across 40 replicate assays inter-assay reproducibility:

Mean Inter-Assay Precision - ≤10% (n= 3 x 40)

Sample	Intra-Assay			Inter-Assay		
	1	2	3	1	2	3
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ppb)	0.466	0.752	1.218	0.451	0.846	1.125
SD	0.030	0.051	0.093	0.030	0.062	0.089
CV (%)	6.4	6.8	7.6	6.7	7.3	7.9

11.3 Cross-Reactivity Rate

Substance	Cross Reactivity Rate
Streptomycin	100%
Dihydrostreptomycin	100%
Kalamycin	< 6.3%
Gentamycin	< 2.5%

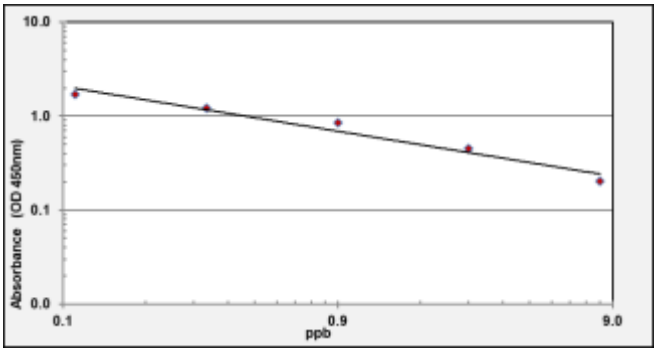
11.4 Recovery

The recovery of Streptomycin spiked at levels throughout the range of the assay in various matrices was evaluated. Samples were diluted prior to assay as directed in the Sample Preparation section.

Sample	Number	Mean Recovery
Tissue	10	85 ± 15%
Milk	10	90 ± 10%
Honey,Royal jelly	10	85 ± 15%
Egg	10	85 ± 15%

11.5Typical Data

For convenience in result calculation, absorbance as abscissa and standard concentrations can be used as ordinate. The standard curve data provided in the manual is only for reference; experimenters should draw the standard curve based on their own data.



ppb	Absorbance		Mean Absorbance	B/B0(%)
	Rep 1	Rep 2		
8.1	0.202	0.199	0.201	9.5
2.7	0.449	0.461	0.455	21.5
0.9	0.843	0.836	0.840	39.7
0.3	1.212	1.297	1.255	59.4
0.1	1.692	1.686	1.689	80.0
0.0	2.103	2.122	2.113	100.0

12. Technical Resources

Technical Support:

For optimal service please be prepared to supply the lot number of the kit used.

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