



Kanamycin ELISA Kit

Item No. 502370

www.caymanchem.com

Customer Service 800.364.9897

Technical Support 888.526.5351

1180 E. Ellsworth Rd · Ann Arbor, MI · USA

TABLE OF CONTENTS

GENERAL INFORMATION	3	Materials Supplied
	4	Safety Data
	4	Precautions
	5	If You Have Problems
	5	Storage and Stability
	5	Materials Needed but Not Supplied
INTRODUCTION	6	Background
	6	About This Assay
	7	Principle of this Assay
	8	Definition of Key Terms
PRE-ASSAY PREPARATION	10	Buffer Preparation
	11	Sample Preparation
	13	Sample Matrix Properties
ASSAY PROTOCOL	18	Preparation of Assay-Specific Reagents
	20	Plate Set Up
	22	Performing the Assay
ANALYSIS	24	Calculations
	26	Performance Characteristics
RESOURCES	31	Troubleshooting
	32	Assay Summary
	33	Plate Template
	34	References
	35	Notes
	35	Warranty and Limitation of Remedy

GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage Temperature
400538	Kanamycin ELISA Antibody	1 vial/100 dtn	-20°C
400537	Kanamycin ELISA-HRP Tracer	1 vial/100 dtn	-20°C
400539	Kanamycin ELISA Standard	1 vial	-20°C
400108	Immunoassay Buffer D Concentrate (5X)	2 vials/10 ml	4°C
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	RT
400035	Polysorbate 20	1 vial/3 ml	RT
400008/400009	Goat Anti-Mouse IgG Coated Plate	1 plate	4°C
400012	96-Well Cover Sheet	1 cover	RT
400040	ELISA Tracer Dye	1 vial	RT
400042	ELISA Antiserum Dye	1 vial	RT
10011355	HRP Stop Solution	1 vial/12 ml	RT
400074	TMB Substrate Solution	2 vials/12 ml	4°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.



WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's Kanamycin ELISA kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

When compared to quantification by LC/MS or GC/MS, it is not uncommon for immunoassays to report higher analyte concentrations. While LC/MS or GC/MS analyses typically measure only a single compound, antibodies used in immunoassays sometimes recognize not only the target molecule, but also structurally related molecules, including biologically relevant metabolites. In many cases, measurement of both the parent molecule and metabolites is more representative of the overall biological response than is the measurement of a short-lived parent molecule. It is the responsibility of the researcher to understand the limits of both assay systems and to interpret their data accordingly.

The stop solution provided with this kit is an acid solution. Please wear appropriate personal protective equipment (e.g. safety glasses, gloves, and lab coat) when using this material.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the Materials Supplied section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm.
2. An orbital microplate shaker
3. Adjustable pipettes; multichannel or repeating pipettor recommended
4. A source of ultrapure water, with a resistivity of 18.2 M Ω -cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
4. Materials used for Sample Preparation (see page 11).

Background

Kanamycin is an aminoglycoside antibiotic.¹⁻³ It is a mixture of several compounds, including its active component and main constituent kanamycin A, minor constituents kanamycin B, C, and D, and under certain conditions, degradation products such as paromamine and 2-deoxystreptamine.¹ Kanamycin binds to the bacterial ribosomal 30S subunit and is active against various Gram-positive and Gram-negative bacteria, including drug-resistant *Mycobacterium tuberculosis*.^{1,3,4} Following administration, kanamycin is rapidly cleared from the periphery but accumulates at high levels in, and is slowly eliminated from, the inner ear and kidney, resulting in eventual oto- and nephrotoxicity, respectively.^{3,4} Formulations containing kanamycin are widely used as dietary supplements for the prevention and treatment of microbial infections in livestock, and this use is associated with enhanced bacterial resistance.¹ In addition, preventative use in livestock induces kanamycin accumulation in animal-derived food products resulting in an increased risk of kanamycin-induced oto- and nephrotoxicity in human consumers.^{1,5} Due to this increased risk of toxicity and the enhancement of antibiotic resistance, it is pertinent to monitor kanamycin levels in food products.

About This Assay

Cayman's Kanamycin ELISA Kit is a competitive assay that can be used for the quantification of kanamycin in milk, egg, and tissue homogenates. The assay has a range of 0.16-100 ng/ml, with a midpoint (50% B/B₀) of 4 ng/ml (4 ppb), and a sensitivity (80% B/B₀) of approximately 1 ng/ml (1 ppb).

Principle of this Assay

This assay is based on the competition between free kanamycin and a kanamycin-HRP conjugate (Kanamycin-HRP Tracer) for a limited number of kanamycin monoclonal antibody binding sites. Because the concentration of the Kanamycin-HRP Tracer is held constant while the concentration of free kanamycin varies, the amount of Kanamycin-HRP Tracer that is able to bind to the Kanamycin Monoclonal Antibody will be inversely proportional to the concentration of free kanamycin in the well. This antibody-kanamycin complex binds to goat anti-mouse IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and TMB Substrate Solution (which contains the substrate to HRP) is added to the well, followed by the HRP Stop Solution. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 450 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of Kanamycin-HRP Tracer bound to the well, which is inversely proportional to the amount of free kanamycin present in the well during the incubation, as described in the equation:

$$\text{Absorbance} \propto [\text{bound kanamycin-HRP tracer}] \propto 1/[\text{kanamycin}]$$

A schematic of this process is shown in Figure 1 on page 8.

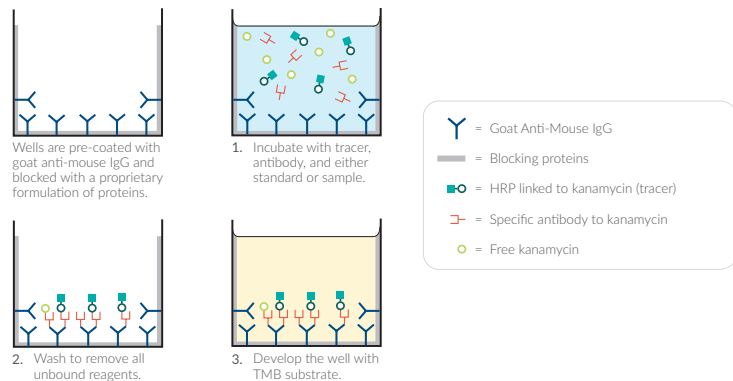


Figure 1. Schematic of the ELISA

Definition of Key Terms

Blk (Blank): background absorbance caused by the TMB Substrate Solution and HRP Stop Solution. The blank absorbance should be subtracted from the absorbance readings of all the other wells, including the non-specific binding (NSB) wells.

TA (Total Activity): total enzymatic activity of the kanamycin HRP-linked tracer.

NSB (Non-Specific Binding): non-immunological binding of the tracer to the well. Even in the absence of specific antibody a very small amount of tracer still binds to the well; the NSB is a measure of this low binding

B₀ (Maximum Binding): maximum amount of the tracer that the antibody can bind in the absence of free analyte.

%B/B₀ (%Bound/Maximum Bound): ratio of the absorbance of a sample or standard well to the average absorbance of the maximum binding (B₀) wells.

Standard Curve: a plot of the %B/B₀ values versus concentration of a series of wells containing various known amounts of analyte.

Dtn (Determination): one dtn is the amount of reagent used per well.

Cross Reactivity: numerical representation of the relative reactivity of this assay towards structurally related molecules as compared to the primary analyte of interest. Biomolecules that possess similar epitopes to the analyte can compete with the assay tracer for binding to the primary antibody. Substances that are superior to the analyte in displacing the tracer result in a cross reactivity that is greater than 100%. Substances that are inferior to the primary analyte in displacing the tracer result in a cross reactivity that is less than 100%. Cross reactivity is calculated by comparing the mid-point (50% B/B₀) value of the tested molecule to the mid-point (50% B/B₀) value of the primary analyte when each is measured in assay buffer using the following formula:

$$\% \text{ Cross Reactivity} = \left[\frac{50\% \text{ B/B}_0 \text{ value for the primary analyte}}{50\% \text{ B/B}_0 \text{ value for the potential cross reactant}} \right] \times 100\%$$

LLOD (Lower Limit of Detection): the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The LLOD is defined as a concentration two standard deviations higher than the mean zero value.

Buffer Preparation

Store all diluted buffers at 4°C; they will be stable for approximately two months.

NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with ultrapure water.

1. Immunoassay Buffer D (1X) Preparation

Dilute the contents of one vial of Immunoassay Buffer D Concentrate (5X) (Item No. 400108) with 40 ml of ultrapure water. Be certain to rinse the vial to remove any salts that may have precipitated.

2. Wash Buffer (1X) Preparation

Dilute the contents of one vial of Wash Buffer Concentrate (400X) (Item No. 400062) with ultrapure water to a total volume of 2 L and add 1 ml of Polysorbate 20. Smaller volumes of Wash Buffer (1X) can be prepared by diluting the Wash Buffer Concentrate (400X) 1:400 and adding 0.5 ml of Polysorbate 20 per 1 L of Wash Buffer (1X).

NOTE: Polysorbate 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.

Sample Preparation

Testing for Interference

This assay has been validated in milk, egg, and tissue homogenates. Other sample types should be tested for interference to evaluate the need for sample purification before embarking on a large number of sample measurements. To test for interference, dilute one or two samples to obtain at least two different dilutions of each sample within the linear portion of the standard curve. If two different dilutions of the same sample show good correlation (differ by 20% or less) in the final calculated kanamycin concentration, sample purification is not required. If you do not see good correlation of the different dilutions, purification is advised. Alternate purification methods will need to be determined by the end user and tested for compatibility in the assay.

Sample Purification Protocol

Milk, egg, and animal tissue samples were purified prior to the assay using the following protocol. Alternative protocols may be used based on the experimental requirements, sample type, and the end user's expertise. Milk samples with higher concentrations of kanamycin (≥ 90 ng/ml) can be tested following dilution with Immunoassay Buffer D (1X), without purification.

Reagents to be prepared prior to purification:

1. Sodium phosphate buffer containing: 150 mM Na_2HPO_4 and 50 mM NaH_2PO_4 in ultrapure water, pH 7.0-7.2.
2. 10% Trichloroacetic acid (TCA): For example, dilute 10 g of TCA in 100 ml of ultrapure water.
3. For tissue homogenates: Homogenize 100 mg of sample in 1 ml of ultrapure water prior to purification.

Sample purification:

1. Aliquot a known amount of each sample into a clean tube (250 μ l is recommended). Vortex the sample to get a uniform mixture.
2. Add 1X the sample volume of 10% TCA.
3. Vortex the sample for 30 seconds and leave at room temperature for 15 minutes.
4. Centrifuge the sample at 12,000 X g at 4°C for 10 minutes.
5. Transfer 200 μ l of the supernatant to a new tube.
6. Neutralize the supernatant with 400 μ l of sodium phosphate buffer and 400 μ l of Immunoassay Buffer D (1X), vortexing at each addition.
7. The sample has now been diluted ten-fold and is ready for ELISA analysis.

General Precautions

- All samples must be free of organic solvents prior to assay.
- Samples should be assayed immediately after collection; milk and egg samples that cannot be assayed immediately should be stored at 4°C; tissue sample should be stored at -20 or -80°C.
- Samples of rodent origin may contain antibodies that interfere with the assay by binding to the goat anti-mouse plate. We recommend that all mouse samples be purified prior to the assay.

Sample Matrix Properties

Parallelism

Milk, egg, and chicken muscle tissue homogenate samples were spiked with different amounts of kanamycin, processed as described in the Sample Preparation section, serially diluted with Immunoassay Buffer D (1X), and evaluated using the Kanamycin ELISA Kit. The results are shown below.

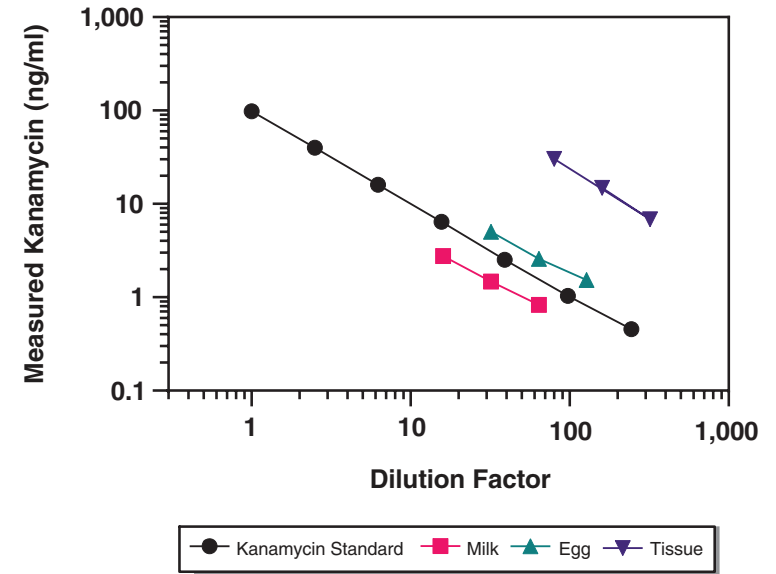


Figure 2. Parallelism of different various matrices in the Kanamycin ELISA

Spike and Recovery

Milk and egg samples were spiked with different amounts of kanamycin, processed as described in the Sample Preparation section, serially diluted with Immunoassay Buffer D (1X), and evaluated using the Kanamycin ELISA Kit. The results are shown below. The error bars represent standard deviations obtained from multiple dilutions of each sample.

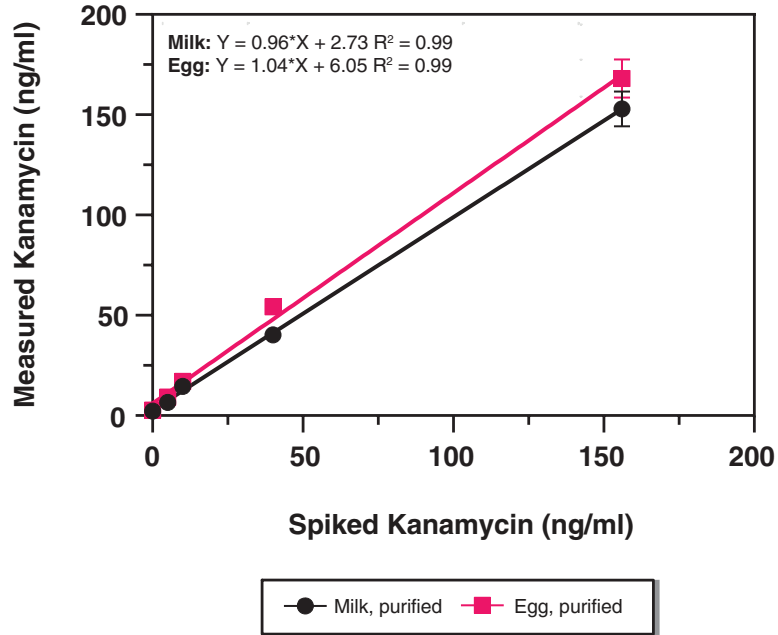


Figure 3. Spike and recovery of Kanamycin in milk and egg

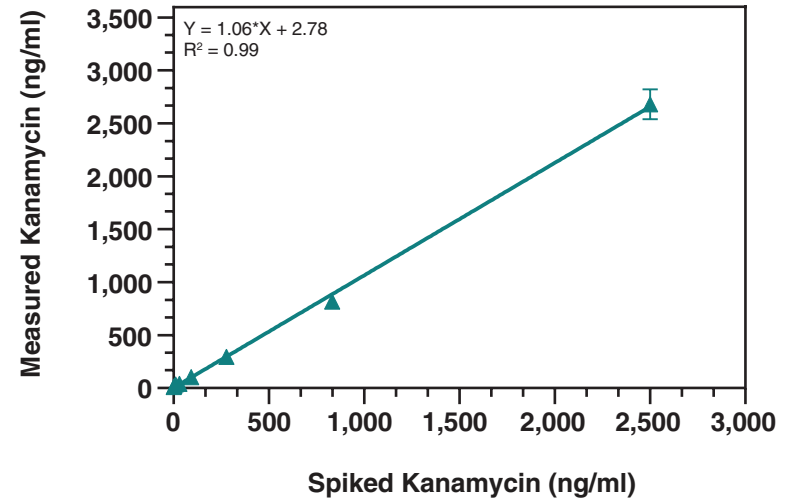


Figure 4. Spike and recovery of Kanamycin in milk without purification

Linearity

Milk, egg, and chicken muscle tissue homogenate samples were spiked with kanamycin, processed as described in the Sample Preparation section, serially diluted with Immunoassay Buffer D (1X), and evaluated for linearity using the Kanamycin ELISA Kit. The results are shown in Table 1 on page 17.

Dilution Factor	Measured Concentration (ng/ml)	Linearity (%)
Milk (spiked with 2,500 ng/ml); diluted		
500	2,478	100
1,000	2,693	109
2,000	2,765	112
Milk (spiked with 40 ng/ml); purified		
16	37.8	100
32	39.4	104
64	43.2	114
Egg (spiked with 10 ng/ml); purified		
16	17.2	100
32	16.8	98
Egg (spiked with 156 ng/ml); purified		
32	167	100
64	159	95
128	178	107
Tissue Homogenate (spiked with 2,500 ng/ml); purified		
160	2,372	100
320	2,192	92

Table 1. Linearity in various matrices

NOTE: Linearity has been calculated using the following formula: %Linearity = (Observed concentration value, dilution adjusted/First observed concentration value in the dilution series, dilution adjusted)*100

Preparation of Assay-Specific Reagents

Kanamycin ELISA Standard

To prepare the standard for use in ELISA: Obtain eight clean test tubes and label them #1-8. Aliquot 900 μ l of Immunoassay Buffer D (1X) in tube #1 and 600 μ l of Immunoassay Buffer D (1X) to tubes #2-8. Equilibrate a pipette tip by repeatedly filling and expelling the tip with the Kanamycin ELISA Standard (Item No. 400539) several times. Using the equilibrated pipette tip, transfer 100 μ l of the standard into tube #1 and mix gently. Serially dilute the standard by removing 400 μ l from tube #1 and placing it in tube #2; mix thoroughly. Next, remove 400 μ l from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-8. These diluted standards should be used within 24 hours.

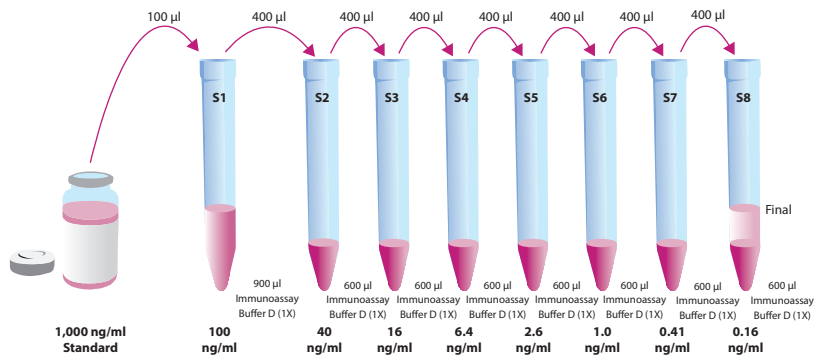


Figure 5. Preparation of the analyte standards

Kanamycin ELISA-HRP Tracer

Dilute the Kanamycin ELISA-HRP Tracer (Item No. 400537) with 5 ml of Immunoassay Buffer D (1X). Store the diluted Kanamycin-HRP Tracer at 4°C (*do not freeze!*). It will be stable for at least 1 week. A 20% surplus of tracer has been included to account for any incidental losses.

Tracer Dye Instructions (optional)

This dye may be added to the tracer, if desired, to aid in visualization of tracer-containing wells. Add the dye to the diluted tracer at a final dilution of 1:100 (add 60 μ l of dye to 6 ml tracer or add 300 μ l of dye to 30 ml of tracer). **NOTE:** Do not store tracer with dye for more than 1 week at 4°C.

Kanamycin ELISA Antibody

Dilute the Kanamycin ELISA Antibody (Item No. 400538) with 5 ml of Immunoassay Buffer D (1X). Store the diluted Kanamycin ELISA Antibody at 4°C (*do not freeze!*). It will be stable for at least 1 week. A 20% surplus of antibody has been included to account for any incidental losses.

Antiserum Dye Instructions (optional)

This dye may be added to the antibody, if desired, to aid in visualization of antibody-containing wells. Add the dye to the diluted antibody at a final dilution of 1:100 (add 60 μ l of dye to 6 ml antibody or add 300 μ l of dye to 30 ml of antibody). **NOTE:** Do not store antibody with dye for more than 24 hours at 4°C.

Plate Set Up

The 96-well plate(s) included with this kit **MUST** be pre-washed five times with Wash Buffer (1X) (300 μ l/well) prior to use in the ELISA. *NOTE: If you do not need to use all the strips at once, place the unwashed strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain a minimum of two Blk, two NSB, and three B_0 wells, and an eight-point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* Each sample should be assayed at a minimum of two dilutions and each dilution should be assayed in duplicate. For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown in Figure 6, on page 21. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see page 24 for more details). We suggest recording the contents of each well on the template sheet provided (see page 33).

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blk	S1	S1	1	1	1	9	9	9	17	17	17
B	Blk	S2	S2	2	2	2	10	10	10	18	18	18
C	NSB	S3	S3	3	3	3	11	11	11	19	19	19
D	NSB	S4	S4	4	4	4	12	12	12	20	20	20
E	B_0	S5	S5	5	5	5	13	13	13	21	21	21
F	B_0	S6	S6	6	6	6	14	14	14	22	22	22
G	B_0	S7	S7	7	7	7	15	15	15	23	23	23
H	TA	S8	S8	8	8	8	16	16	16	24	24	24

Blk - Blank
TA - Total Activity
NSB - Non-Specific Binding
 B_0 - Maximum Binding
S1-S8 - Standards 1-8
1-24 - Samples

Figure 6. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Pre-Wash the Plate

Rinse the plates (or strips to be used) five times with ~300 μ l of Wash Buffer (1X).

Addition of the Reagents

1. Immunoassay Buffer D (1X)

Add 100 μ l of Immunoassay Buffer D (1X) to NSB wells. Add 50 μ l of Immunoassay Buffer D (1X) to B₀ wells.

2. Kanamycin ELISA Standard

Add 50 μ l from tube #8 to both of the lowest standard wells (S8). Add 50 μ l from tube #7 to each of the next standard wells (S7). Continue with this procedure until all the standards are aliquoted. The same pipette tip should be used to aliquot all the standards. Before pipetting each standard, be sure to equilibrate the pipette tip in that standard.

3. Samples

Add 50 μ l of sample per well. Each sample should be assayed at a minimum of two dilutions. Each dilution should be assayed in duplicate (triplicate recommended).

4. Kanamycin-HRP Tracer

Add 50 μ l to each well except the TA and Blk wells.

5. Kanamycin ELISA Antibody

Add 50 μ l to each well, except the TA, NSB, and Blk wells, within 15 minutes of addition of the tracer.

Incubation of the Plate

Cover each plate with a 96-Well Cover Sheet (Item No. 400012) and incubate one hour at room temperature on an orbital shaker.

Development of the Plate

1. Empty the wells and rinse five times with ~300 μ l of Wash Buffer (1X).
2. Add 175 μ l of TMB Substrate Solution (Item No. 400074) to each well.
3. Dilute 10 μ l of the previously diluted tracer with 90 μ l of Immunoassay Buffer D (1X). Add 5 μ l of this solution to the TA wells.
4. Cover the plate with the 96-Well Cover Sheet and protect from light. Optimum development is obtained by using an orbital shaker at RT for 30 minutes.
5. Remove the cover sheet being careful to keep TMB Substrate Solution from splashing on the cover. *NOTE: Any loss of TMB Substrate Solution will affect the absorbance readings.*
6. **DO NOT WASH THE PLATE.** Add 75 μ l of HRP Stop Solution (Item No. 10011355) to each well of the plate. Blue wells should turn yellow and colorless wells should remain colorless. *NOTE: The stop solution in this kit contains an acid. Wear appropriate protection and use caution when handling this solution.*

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Read the plate at a wavelength of 450 nm.

ANALYSIS

Many plate readers come with data reduction software that plots data automatically. Alternatively a spreadsheet program can be used. The data should be plotted as either %B/B₀ versus log concentration using a four-parameter logistic fit or as logit B/B₀ versus log concentration using a linear fit. **NOTE: Cayman has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/elisa) to obtain a free copy of this convenient data analysis tool.**

Calculations

Preparation of the Data

The following procedure is recommended for preparation of the data prior to graphical analysis.

NOTE: If the plate reader has not subtracted the absorbance readings of the blank wells from the absorbance readings of the rest of the plate, be sure to do that now.

1. Average the absorbance readings from the NSB wells.
2. Average the absorbance readings from the B₀ wells.
3. Subtract the NSB average from the B₀ average. This is the corrected B₀ or corrected maximum binding.
4. Calculate the B/B₀ (Sample or Standard Bound/Maximum Bound) for the remaining wells. To do this, subtract the average NSB absorbance from the S1 absorbance and divide by the corrected B₀ (from Step 3). Repeat for S2-S8 and all sample wells. (To obtain %B/B₀ for a logistic four-parameter fit, multiply these values by 100.)

NOTE: The TA values are not used in the standard curve calculations. Rather, they are used as a diagnostic tool. Low or no absorbance from a TA well could indicate a dysfunction in the enzyme-substrate system.

Plot the Standard Curve

Plot %B/B₀ for standards S1-S8 versus kanamycin concentration using linear (y) and log (x) axes and perform a 4-parameter logistic fit.

Alternative Plot - The data can also be linearized using a logit transformation. The equation for this conversion is shown below. **NOTE: Do not use %B/B₀ in this calculation.**

$$\text{logit (B/B}_0\text{)} = \ln [\text{B/B}_0\text{}/(1 - \text{B/B}_0\text{)}]$$

Plot the data as logit (B/B₀) versus log concentrations and perform a linear regression fit.

Determine the Sample Concentration

Calculate the B/B₀ (or %B/B₀) value for each sample. Determine the concentration of each sample by identifying the %B/B₀ on the standard curve and reading the corresponding values on the x-axis. **NOTE: Remember to account for any dilution of the sample prior to the addition to the well.** If samples were purified using the protocol described on page 11, multiply the ELISA results by the dilution factor of 10. Samples with %B/B₀ values greater than 80% or less than 20% should be re-assayed as they generally fall out of the linear range of the standard curve. A 20% or greater disparity between the apparent concentration of two different dilutions of the same sample indicates interference which could be eliminated by purification.

1 ng/ml of kanamycin = 1 ppb

Performance Characteristics

Sample Data

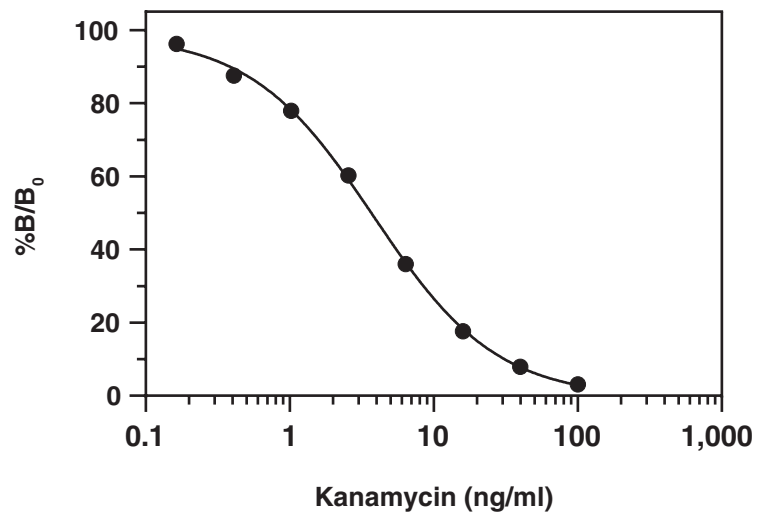
The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You **must** run a new standard curve. Do not use the data below to determine the value of your samples. Your results could differ substantially.

Kanamycin Standards (ng/ml) and Controls	Blk-Subtracted Absorbance	NSB-Corrected Absorbance	%B/B ₀	%CV* Itra-Assay Precision	%CV* Inter-Assay Precision
NSB	0.013	--	--	--	--
B ₀	2.124	--	--	--	--
TA	0.659	0.646	--	--	--
100	0.079	0.066	3.1	23.6**	23.3**
40	0.180	0.167	7.9	7.3	8.3
16	0.386	0.373	17.7	3.3	2.0
6.4	0.774	0.761	36.1	6.9	1.7
2.6	1.284	1.271	60.2	6.1	3.1
1.0	1.659	1.646	78.0	13.1	10.8
0.41	1.862	1.849	87.6	35.6**	10.8
0.16	2.045	2.032	96.3	56.5**	19.8

Table 2. Typical results

*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve

**Evaluate data in this range with caution



Assay Range = 0.16-100 ng/ml
Sensitivity (defined as 80% B/B₀) = 1.0 ng/ml
Mid-point (defined as 50% B/B₀) = 3.9 ng/ml
Lower Limit of Detection (LLOD) = 0.29 ng/ml
 The sensitivity and mid-point were derived from the standard curve shown above. The standards were diluted with Immunoassay Buffer D (1X).

Figure 7. Typical standard curve

Precision:

Intra-assay precision was determined by analyzing 24 replicates of three kanamycin controls (spiked milk without purification) in a single assay.

Matrix Control (ng/ml)	%CV
545	5.1
166	7.9
67.5	4.2

Table 3. Intra-assay variation

Inter-assay precision was determined by analyzing three kanamycin controls (spiked milk without purification) in nine separate assays on three different days.

Matrix Control (ng/ml)	%CV
516	15.7
174	17.3
65.0	13.7

Table 4. Inter-assay variation

Cross Reactivity:

Compound	Cross Reactivity
Kanamycin	100%
Streptomycin	<0.01%
G418	<0.01%
Hygromycin B	<0.01%
Gentamicin	<0.01%
Dihydrostreptomycin	<0.01%
Neomycin	<0.01%
Apramycin	<0.01%
Paromomycin	<0.01%
Amikacin	<0.01%
Tetracycline	<0.01%
Ampicillin	<0.01%

Table 5. Cross Reactivity of the kanamycin ELISA

RESOURCES

Troubleshooting

Problem	Possible Causes
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique
High NSB (>0.100)	A. Poor washing B. Exposure of NSB wells to specific kanamycin antibody
Very low B ₀	A. Trace organic contaminants in the water source B. Dilution error in preparing reagents
Low sensitivity (shift in dose response curve)	A. Standard is degraded or contaminated B. Dilution error in preparing reagents
Analyses of two dilutions of a biological sample do not agree (<i>i.e.</i> , more than 20% difference)	Interfering substances are present
Low signal in the sample wells (below the range of the standard curve)	A. HRP inhibitors are present; ensure that the samples and buffers are free of HRP inhibitors, such as azide B. Sample requires further dilution
Only Total Activity (TA) wells develop	Trace organic contaminants in the water source

Procedure	Blk	TA	NSB	B ₀	Standards/ Samples
Plate Preparation	Wash the plates or strips to be used for the assay 5 times with ~300 µl of Wash Buffer (1X)				
Dilute and Mix	Mix all reagents gently				
Add Immunoassay Buffer D (1X)	--	--	100 µl	50 µl	--
Add Standards/Samples	--	--	--	--	50 µl
Add Kanamycin-HRP Tracer	--	--	50 µl	50 µl	50 µl
Add Kanamycin ELISA Antibody	--	--	--	50 µl	50 µl
Incubate	Seal the plate and incubate for 1 hour at room temperature on an orbital shaker				
Aspirate	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)				
Add TMB Substrate Solution	Add 175 µl of TMB Substrate Solution				
TA - Add Tracer diluted 1:10	--	5 µl	--	--	--
Incubate	Seal the plate and incubate for 30 minutes at room temperature on an orbital shaker protected from light				
Add HRP Stop Solution	75 µl				
Read	Read absorbance at 450 nm				

Table 6. Assay Summary

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H

References

1. Zhang, X., Wang, J., Wu, Q., *et al.* Determination of kanamycin by high performance liquid chromatography. *Molecules* **24(10)**, 1902 (2019).
2. Hotta, K. and Kondo, S. Kanamycin and its derivative, arbekacin: Significance and impact. *J. Antibiot.* **71(4)**, 417-424 (2018).
3. Pindell, M.H. The pharmacology of kanamycin—a review and new developments. *Ann. N. Y. Acad. Sci.* **132(2)**, 805-810 (1966).
4. Toyoda, Y. and Tachibana, M. Tissue levels of kanamycin in correlation with oto- and nephrotoxicity. *Acta Otolaryngol.* **86(1-2)**, 9-14 (1978).
5. Wang, B.B., Zhao, X., Chen, L.-J., *et al.* Functionalized persistent luminescence nanoparticle-based aptasensor for autofluorescence-free determination of kanamycin in food samples. *Anal. Chem.* **93(4)**, 2589-2595 (2021).

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

This document is copyrighted. All rights are reserved. This document may not, in whole or part, be copied, photocopied, reproduced, translated, or reduced to any electronic medium or machine-readable form without prior consent, in writing, from Cayman Chemical Company.

©07/19/2023, Cayman Chemical Company, Ann Arbor, MI, All rights reserved.
Printed in U.S.A.

