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# Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

## Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

## Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand


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# 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

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# 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 MΩ·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).

# INTRODUCTION

## 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% $B/B_0$) of 4 ng/ml (4 ppb), and a sensitivity (80% $B/B_0$) of approximately 1 ng/ml (1 pbb).



## 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.

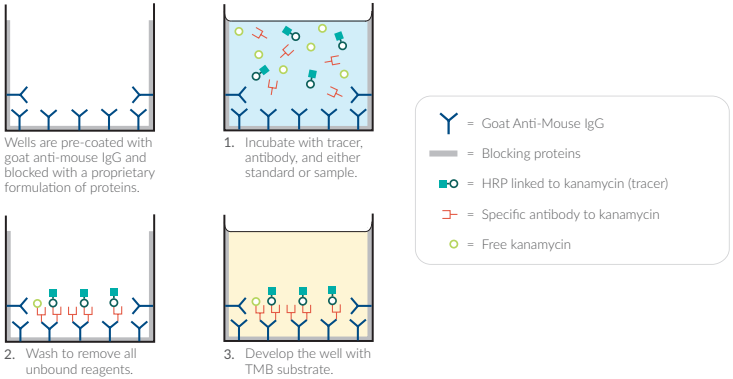



**Figure 1. Schematic of the ELISA**

## 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

**$B_0$ (Maximum Binding):** maximum amount of the tracer that the antibody can bind in the absence of free analyte.

**%$B/B_0$ (%Bound/Maximum Bound):** ratio of the absorbance of a sample or standard well to the average absorbance of the maximum binding ($B_0$) wells.

**Standard Curve:** a plot of the %$B/B_0$ 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% $B/B_0$) value of the tested molecule to the mid-point (50% $B/B_0$) value of the primary analyte when each is measured in assay buffer using the following formula:

$$\text{\% Cross Reactivity} = \left[ \frac{\text{50\% } B/B_0 \text{ value for the primary analyte}}{\text{50\% } B/B_0 \text{ value for the potential cross reactant}} \right] \text{x } 100\%$$

**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.

# PRE-ASSAY PREPARATION

## 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 µl 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 µl of the supernatant to a new tube.
6. Neutralize the supernatant with 400 µl of sodium phosphate buffer and 400 µl 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.

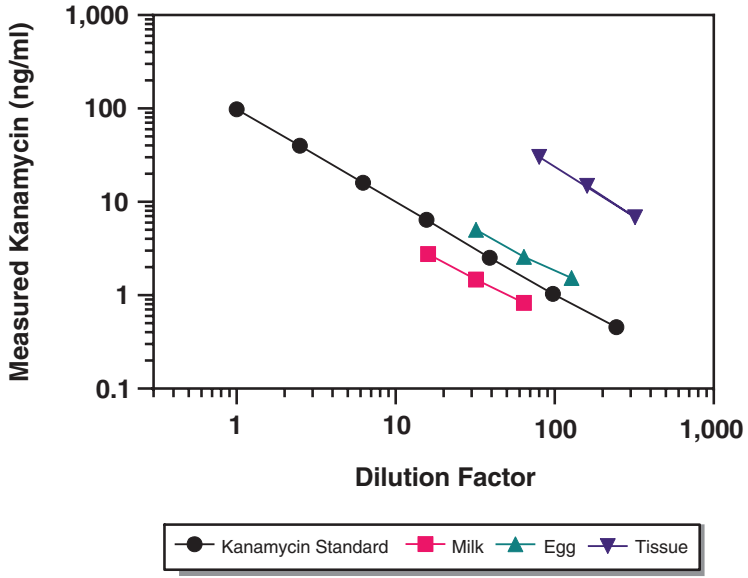



**Figure 2. Parallelism of different various matrices in the Kanamycin ELISA**

## 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.

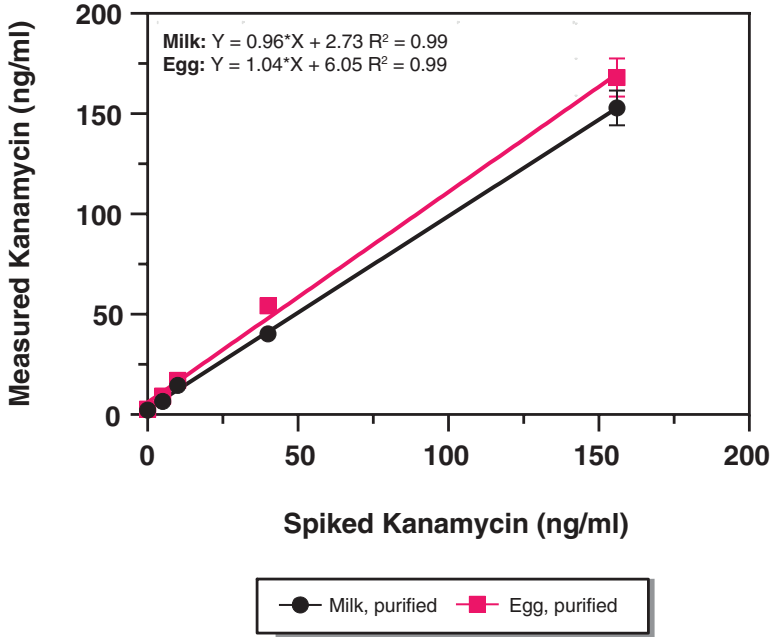



**Figure 3. Spike and recovery of Kanamycin in milk and egg**

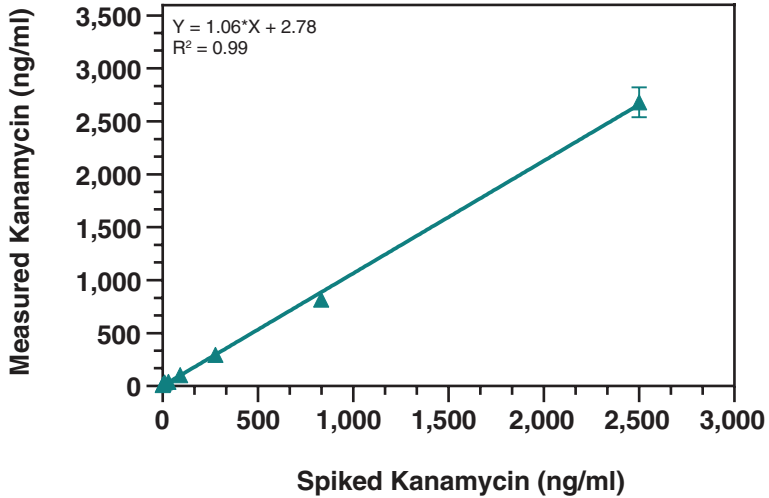



**Figure 4. Spike and recovery of Kanamycin in milk without purification**

## 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*

# ASSAY PROTOCOL

## 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 µl of Immunoassay Buffer D (1X) in tube #1 and 600 µl 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 µl of the standard into tube #1 and mix gently. Serially dilute the standard by removing 400 µl from tube #1 and placing it in tube #2; mix thoroughly. Next, remove 400 µl 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.

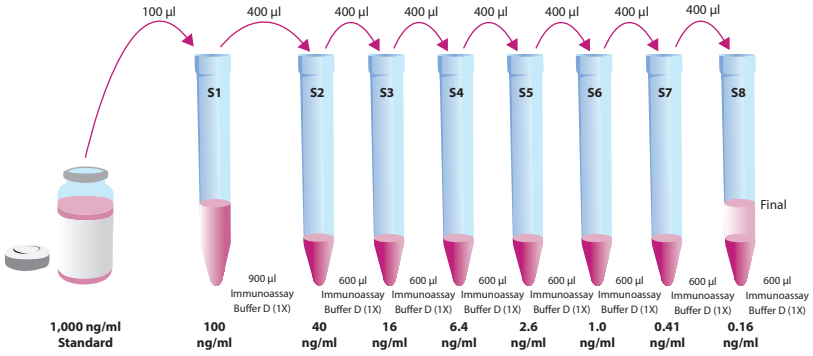



**Figure 5. Preparation of the analyte standards**



### 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 µl of dye to 6 ml tracer or add 300 µl 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)**

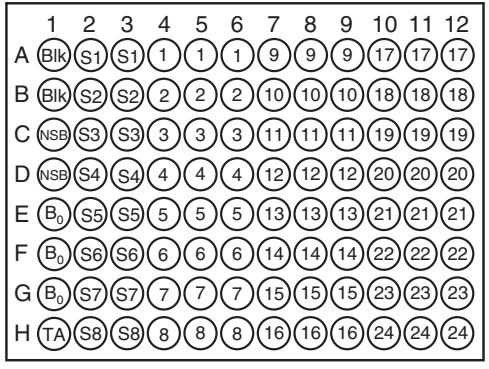
This dye may be added to the antibody, if desired, to aid in visualization of antibody-containing wells. Add the dye to the diluted antibody at a final dilution of 1:100 (add 60 µl of dye to 6 ml antibody or add 300 µl of dye to 30 ml of antibody). *NOTE: Do not store antibody with dye for more than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




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 µl of Wash Buffer (1X).

### Addition of the Reagents

1. **Immunoassay Buffer D (1X)**

   Add 100 µl of Immunoassay Buffer D (1X) to NSB wells. Add 50 µl of Immunoassay Buffer D (1X) to $B_0$ wells.

2. **Kanamycin ELISA Standard**

   Add 50 µl from tube #8 to both of the lowest standard wells (S8). Add 50 µl 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 µl 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 µl to each well except the TA and Blk wells.

5. **Kanamycin ELISA Antibody**

   Add 50 µl 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 µl of Wash Buffer (1X).
2. Add 175 µl of TMB Substrate Solution (Item No. 400074) to each well.
3. Dilute 10 µl of the previously diluted tracer with 90 µl of Immunoasasy Buffer D (1X). Add 5 µl 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 µl 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/$B_0$ *versus* log concentration using a four-parameter logistic fit or as logit B/$B_0$ *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 $B_0$ wells.
3. Subtract the NSB average from the $B_0$ average. This is the corrected $B_0$ or corrected maximum binding.
4. Calculate the B/$B_0$ (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 $B_0$ (from Step 3). Repeat for S2-S8 and all sample wells. (To obtain %B/$B_0$ 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/$B_0$ 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 lineraized using a logit transformation. The equation for this conversion is shown below. *NOTE: Do not use %B/$B_0$ in this calculation.*

$$\text{logit } (B/B_0) = \ln [B/B_0/(1 - B/B_0)]$$

Plot the data as logit (B/$B_0$) *versus* log concentrations and perform a linear regression fit.

### Determine the Sample Concentration

Calculate the B/$B_0$ (or %B/$B_0$) value for each sample. Determine the concentration of each sample by identifying the %B/$B_0$ 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/$B_0$ 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

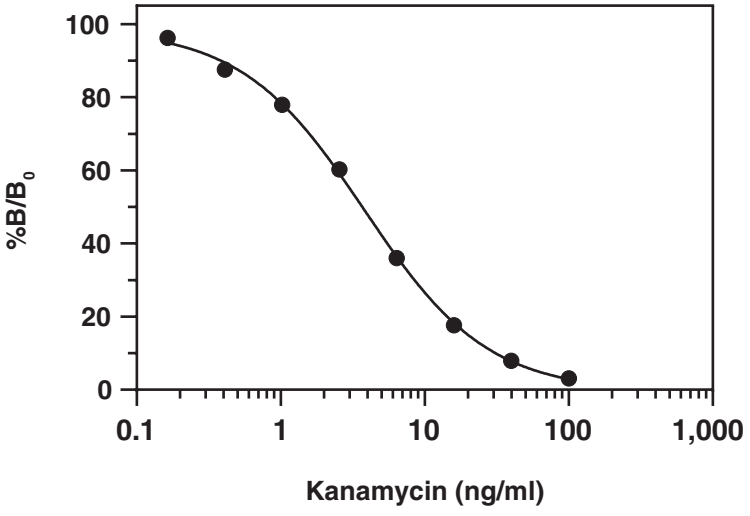
The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve. Do not use the data below to determine the value of your samples. Your results could differ substantially.

| Kanamycin Standards (ng/ml) and Controls | Blk-Substracted 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% $B/B_0$) = 1.0 ng/ml
**Mid-point** (defined as 50% $B/B_0$) = 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**



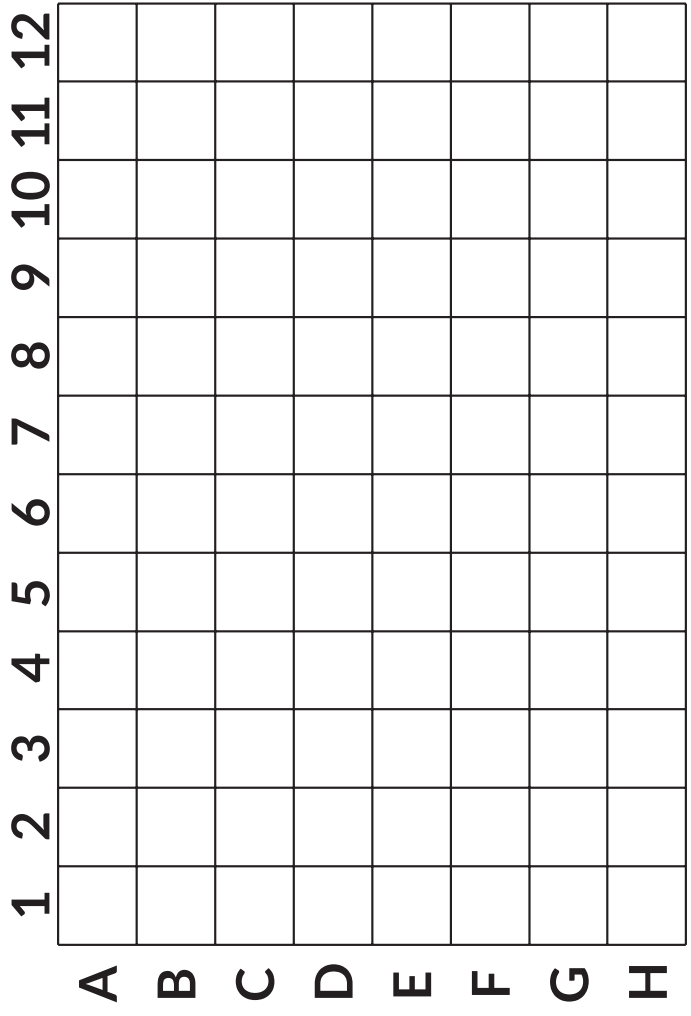
# RESOURCES

## Troubleshooting

| Problem | Possible Causes |
|---|---|
| Erratic values; dispersion of duplicates | A. Trace organic contaminants in the water source<br>B. Poor pipetting/technique |
| High NSB (>0.100) | A. Poor washing<br>B. Exposure of NSB wells to specific kanamycin antibody |
| Very low $B_0$ | A. Trace organic contaminants in the water source<br>B. Dilution error in preparing reagents |
| Low sensitivity (shift in dose response curve) | A. Standard is degraded or contaminated<br>B. Dilution error in preparing reagents |
| Analyses of two dilutions of a biological sample do not agree (*i.e.*, 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<br>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**



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## NOTES

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