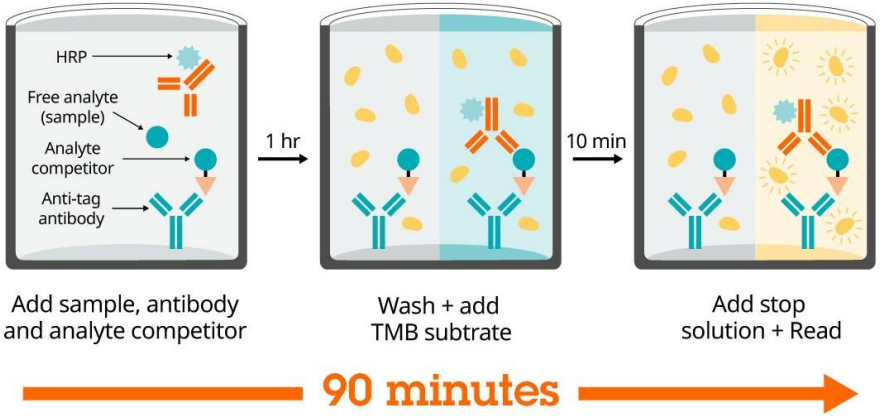
ab318951 – Kanamycin SimpleStep ELISA® Kit – Intracellular

For the quantitative measurement of Kanamycin in mouse, chicken, pork, and shrimp tissue extracts.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab318951



Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Materials Supplied

Item	Quantity 1 x 96 tests	Quantity 10 x 96 tests	Storage Condition
Kanamycin CaptSure™ Conjugate 50X	80 µL	10 x 80 µL	+4°C
Kanamycin HRP Conjugate 50X	80 µL	10 x 80 µL	+4°C
Kanamycin Lyophilized Standard	2 Vials	10 x 2 Vials	+4°C
Antibody Diluent 4BR	8 mL	10 x 8 mL	+4°C
Cell Extraction Buffer PTR 5X	10 mL	2 x 50mL	+4°C
Sample Diluent NS*	12 mL	-	+4°C
Wash Buffer PT 10X	20 mL	200 mL	+4°C
TMB Development Solution	12 mL	120 mL	+4°C
Stop Solution	12 mL	120 mL	+4°C
SimpleStep Pre-Coated 96-Well Microplate	96 wells	10 x 96 wells	+4°C
Plate Seal	1	10	+4°C

*Sample Diluent NS is provided but not necessary for this product

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Microplate reader capable of measuring absorbance at 450 or 600 nm.
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Plate shaker for all incubation steps.
Method for determining total protein concentration in sample (BCA assay recommended).
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. The CaptSure and HRP Conjugates have only been tested for stability in the provided 50X formulation.

1X Cell Extraction Buffer PTR (For cell and tissue extracts only): Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 8 mL deionized water and 2 mL Cell Extraction Buffer PTR 5X. Mix thoroughly and gently. If required protease inhibitors can be added.

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

CaptSure Conjugate Solution: Prepare CaptSure Conjugate Solution by diluting the 50X CaptSure Conjugate in Antibody Diluent 4BR. To make 2 mL of the Capture Conjugate Solution combine 40 µL 50X CaptSure Conjugate with 1.96 mL Antibody Diluent 4BR. Mix thoroughly and gently.

HRP Conjugate Solution: Prepare HRP Conjugate Solution by diluting the 50X HRP Conjugate in Antibody Diluent 4BR. To make 2 mL of the HRP Conjugate Solution combine 40 µL 50X HRP Conjugate with 1.96 mL Antibody Diluent 4BR. Mix thoroughly and gently.

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. Reconstitute the Kanamycin standard sample by adding the volume of 1X Cell Extraction Buffer PTR indicated on the standard vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 20,000 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 252 µL of 1X Cell Extraction Buffer PTR into tube number 1 and 180 µL of 1X Cell Extraction Buffer PTR into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no standard and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	108	252	20,000	6,000
2	Standard#1	180	180	6,000	3,000
3	Standard#2	180	180	3,000	1,500
4	Standard#3	180	180	1,500	750
5	Standard#4	180	180	750	375
6	Standard#5	180	180	375	187.5
7	Standard#6	180	180	187.5	93.75
8	Blank Control	0	180	N/A	N/A

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Mouse Kidney Extract	0.0313 – 0.25 mg/mL
Chicken Extract	0.125 – 1 mg/mL
Pork Extract	0.125 – 1 mg/mL
Shrimp Extract	0.125 – 1 mg/mL

Preparation of extracts from tissue homogenates Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (Dounce homogenizer recommended). Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
For each assay performed, a minimum of two wells must be used as the zero control.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
Differences in well absorbance or "edge effects" have not been observed with this assay.

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls, and samples in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
3. Add 60 µL of all sample or standard to appropriate wells.
4. Add 30 µL of the CaptSure Conjugate Solution to each well.
    a. Optional – for non-specific binding wells, add 30 µL of Antibody Diluent 4BR in lieu of CaptSure Conjugate Solution.
5. Add 30 µL of the HRP Conjugate Solution to each well.
6. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.
7. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 30 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
8. Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.
   Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.
   Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.
9. Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.
10. Alternative to 8 – 9: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips: www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of Kanamycin.

The standard in this kit is free Kanamycin.

Spike experiments were used to validate tissue extract.

Serum, plasma (heparin), plasma (EDTA), plasma (citrate), cell culture supernatant, saliva, urine, milk, and CSF samples have not been tested with this kit.

This kit is incompatible with cell extract samples.

For the measurement of Kanamycin in serum, plasma, cell culture supernatant, urine, and milk, use Kanamycin ELISA kit ab318950.

CROSS REACTIVITY

Cross reactivity was determined for related compounds at 4,000 and 40,000 pg/mL. Cross reactivity is reported as percent interpolated concentration relative to Kanamycin.

Compound	Cross Reactivity – 4,000 pg/mL (%)	Cross Reactivity – 40,000 pg/mL (%)
Kanamycin	100	-
Gentamicin	0	2
Neomycin	0	0
Streptomycin	0	0

INTERFERENCE

4,000 and 40,000 pg/mL of Gentamicin, Neomycin, and Streptomycin were tested for interference with 500 pg/mL of Kanamycin. No interference was observed.

SPECIES REACTIVITY

Validated in mouse, chicken, pork, and shrimp samples, reactivity is species independent.

CALCULATION

- Optional: Non-specific binding (NSB) well subtracted values can be calculated by averaging the absorbance values for the NSB wells and subtracting the average NSB absorbance value from all other absorbance values.
- Create a standard curve by plotting the average absorbance value for each standard concentration (y-axis) against the target concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.
- Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target analyte in the sample by interpolating absorbance values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target analyte in the sample.
- Samples generating absorbance values lower than that of the highest concentration standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance value greater than that of the lowest concentration standard should be retested in a less dilute form.
- Optional: The binding percentage, B/B_0, can be calculated by dividing the average absorbance value for each standard or sample by the average absorbance of the zero standard (B_0).

TYPICAL DATA

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Standard Curve Measurements				
Concentration (pg/mL)	O.D. 450 nm		Mean O.D.	B/B_0 (%)
	1	2		
NSB	0.037	0.036	0.037	-
0	1.531	1.530	1.531	100%
93.75	1.263	1.254	1.259	82%
187.5	1.002	1.009	1.005	66%
375	0.845	0.799	0.822	54%
750	0.567	0.573	0.570	37%
1,500	0.356	0.353	0.354	23%
3,000	0.218	0.222	0.220	14%
6,000	0.126	0.126	0.126	8%

Table 1. Example of Kanamycin standard curve in 1X Cell Extraction Buffer PTR. The Kanamycin standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The calculated minimal detectable dose (MDD) is 33.7 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=24) and subtracting 2 standard deviations then extrapolating the corresponding concentration.

Recovery

3 concentrations of Kanamycin were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
1 mg/mL Chicken Extract	87	85 – 90%
1 mg/mL Pork Extract	93	87 – 100%
1 mg/mL Shrimp Extract	107	105 – 110%
0.25 mg/mL Mouse Kidney Extract	103	96 – 108%

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Kanamycin was spiked in in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Cell Extraction Buffer PTR.

Dilution Factor	Interpolated value	1 mg/mL Chicken Extract	1 mg/mL Pork Extract	1 mg/mL Shrimp Extract	0.25 mg/mL Mouse Kidney Extract
Undiluted	pg/mL	1,985	1,734	2,333	1,694
	% Expected value	100	100	100	100
2	pg/mL	1,073	987	1,228	737
	% Expected value	108	114	105	87
4	pg/mL	512	462	563	346
	% Expected value	103	107	96	82
8	pg/mL	244	225	282	173
	% Expected value	98	104	97	82

Precision

Mean coefficient of variations of interpolated values of Kanamycin from 1 mg/mL Pork Extract spiked with Kanamycin within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	6.3	2.5

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
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