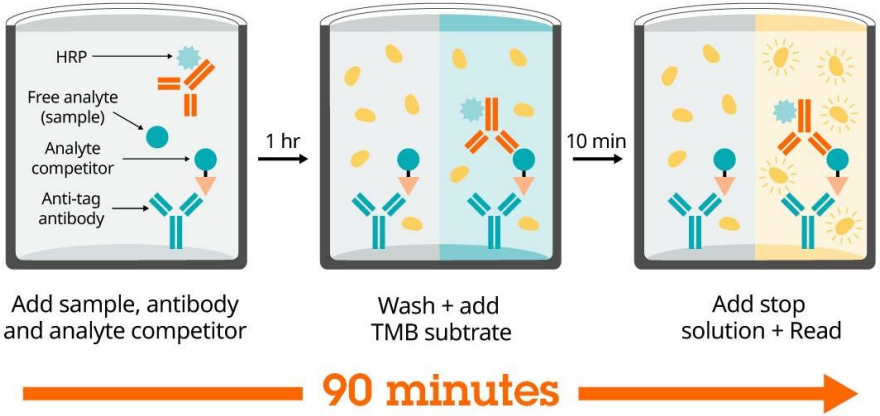
ab318950 – Kanamycin SimpleStep ELISA® Kit – Extracellular

For the quantitative measurement of Kanamycin in human and serum, plasma (citrate), plasma (EDTA), plasma (heparin), urine, milk, and cell culture supernatant.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab318950



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
Sample Diluent NS	12 mL	100 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

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.
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 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 Sample Diluent NS 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 Sample Diluent NS into tube number 1 and 180 µL of Sample Diluent NS 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**
Serum	2.5 – 20%
Plasma – Citrate	2.5 – 20%
Plasma – EDTA	2.5 – 20%
Plasma – Heparin	2.5 – 20%
Urine	2.5 – 20%
Milk (Bovine)	1.25 – 10%
Cell Culture Supernatant	6.25 – 50%

Serum Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples at least 1:10 into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1:10 into Sample Diluent NS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Urine Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Dilute samples at least 1:10 into Sample Diluent NS and assay. Store un-diluted urine samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Milk De-fat milk samples as follows. Centrifuge milk samples at 500 x g for 15 minutes at 4°C and collect the aqueous fraction using syringe attached to needle. Centrifuge the aqueous fraction at 3,000 x g for 15 minutes at 4°C and collect the final aqueous fraction (de-fatted milk) using syringe attached to needle. Dilute samples at least 1:10 into Sample Diluent NS and assay. Store un-diluted de-fatted milk at -20°C or below. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants. Dilute samples at least 1:1 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

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.

Spiked experiments were used to validate serum, plasma (heparin), plasma (EDTA), plasma (citrate), cell culture supernatant, urine, and milk.

Saliva, CSF, cell extract, and tissue extract samples have not been tested with this kit.

Assay may be compatible with alternate diluents. To test alternate sample diluents, prepare the standard curve in the alternate diluent as well.

For the measurement of Kanamycin in tissue extracts use Kanamycin ELISA kit ab318951.

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	3
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 Human and Mouse 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 (B_0)	1.694	1.692	1.693	100%
93.75	1.441	1.422	1.431	85%
187.5	1.154	1.149	1.152	68%
375	0.808	0.836	0.822	49%
750	0.579	0.608	0.593	35%
1,500	0.362	0.365	0.364	21%
3,000	0.207	0.213	0.210	12%
6,000	0.117	0.121	0.119	7%

Table 1. Example of Kanamycin standard curve in Sample Diluent NS. 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 38.3 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 (%)
20% Human Serum	105	103 – 107
20% Human Plasma – Citrate	105	101 – 110
20% Human Plasma – EDTA	111	110 – 111
20% Human Plasma - Heparin	104	101 – 107
20% Mouse Serum	112	108 – 118
20% Mouse Plasma – Citrate	103	94 – 109
20% Mouse Plasma – EDTA	110	107 – 112
20% Mouse Plasma - Heparin	112	107 – 119
20% Human Urine	102	94 – 117
10% Bovine Milk	108	102 – 119
50% Cell Culture Media*	91	83 – 97

*Media is DMEM containing 10% fetal calf serum

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 Sample Diluent NS.

Dilution Factor	Interpolated value	20% Human Serum	20% Human Plasma (Citrate)	20% Human Plasma (EDTA)	20% Human Plasma (Heparin)
Undiluted	pg/mL	2,275	2,177	2,495	2,368
	% Expected value	100	100	100	100
2	pg/mL	1,079	1,130	1,176	1,217
	% Expected value	95	104	94	103
4	pg/mL	552	560	631	553
	% Expected value	97	103	101	93
8	pg/mL	294	293	280	259
	% Expected value	103	108	90	88

Dilution Factor	Interpolated value	20% Mouse Serum	20% Mouse Plasma (Citrate)	20% Mouse Plasma (EDTA)	20% Mouse Plasma (Heparin)
Undiluted	pg/mL	2,392	2,201	2,215	2,185
	% Expected value	100	100	100	100
2	pg/mL	1,309	1,037	1,094	1,125
	% Expected value	109	94	99	103
4	pg/mL	656	516	625	562
	% Expected value	110	94	113	103
8	pg/mL	253	228	287	276
	% Expected value	85	83	104	101

Dilution Factor	Interpolated value	20% Human Urine	10% Bovine Milk	50% Cell Culture Media*
Undiluted	pg/mL	2,127	1,573	1,651
	% Expected value	100	100	100
2	pg/mL	994	860	924
	% Expected value	93	109	112
4	pg/mL	486	472	465
	% Expected value	91	120	113
8	pg/mL	237	177	234
	% Expected value	89	90	113

*Media is DMEM containing 10% fetal calf serum

Precision

Mean coefficient of variations of interpolated values of Kanamycin from 10% bovine milk spiked with Kanamycin within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	6.3	9.8

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