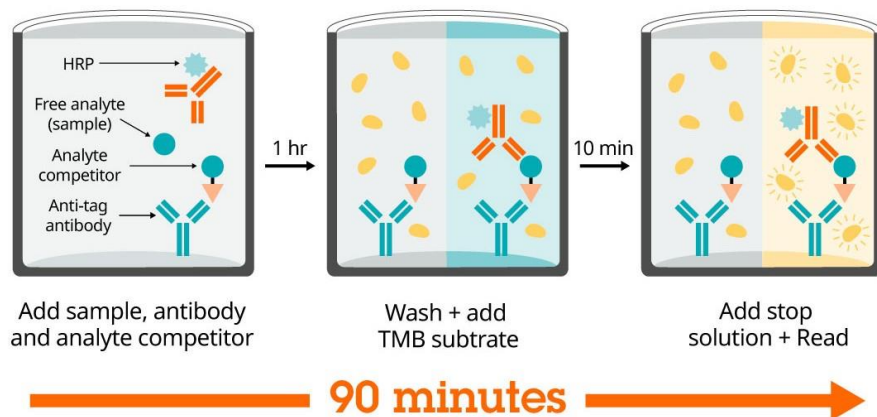


ab322084 – Gentamicin SimpleStep ELISA® Kit – Extracellular

For the quantitative measurement of Gentamicin in serum, plasma (citrate), plasma (EDTA), 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/ab322084



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
Gentamicin CaptSure™ Conjugate 50X	80 µL	10 x 80 µL	+4°C
Gentamicin HRP Conjugate 50X	80 µL	10 x 80 µL	+4°C
Human Gentamicin Lyophilized Standard	2 Vials	10 x 2 Vials	+4°C
Antibody Diluent 4BI	8 mL	10 x 8 mL	+4°C
Cell Extraction Buffer SSW	10 mL	2 X 50 mL	+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.

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.

1X Cell Extraction Buffer SSW: Prepare 1X Cell Extraction Buffer SSW by diluting Cell Extraction Buffer SSW Stock to 1X with 1X Wash Buffer PT. To make 10 mL 1X Cell Extraction Buffer SSW combine 9.6 mL 1X Wash Buffer PT and 0.4 mL Cell Extraction Buffer SSW Stock. Mix thoroughly and gently. If required protease inhibitors can be added.

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

HRP Conjugate Solution: Prepare HRP Conjugate Solution by diluting the 50X HRP Conjugate in Antibody Diluent 4BI. To make 2 mL of the HRP Conjugate Solution combine 40 µL 50X HRP Conjugate with 1.96 mL Antibody Diluent 4BI. 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 Gentamicin standard sample by adding the volume of 1X Cell Extraction Buffer SSW 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 – 7.
3. Add 324 µL of 1X Cell Extraction Buffer SSW into tube number 1 and 180 µL of 1X Cell Extraction Buffer SSW into numbers 2 – 7.
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	36	324	20,000	2,000
2	Standard#1	180	180	2,000	1,000
3	Standard#2	180	180	1,000	500
4	Standard#3	180	180	500	250
5	Standard#4	180	180	250	125
6	Standard#5	180	180	125	62.5
7	Blank Control	0	180	N/A	N/A

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	Range
Serum	1.25 – 10%
Plasma – Citrate	1.25 – 10%
Plasma – EDTA	1.25 – 10%
Urine	1.25 – 10%
Milk	1.25 – 10%
Cell Culture Supernatant	3.125 – 25%

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 1X Cell Extraction Buffer SSW and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using citrate or EDTA. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1:10 into 1X Cell Extraction Buffer SSW 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 1X Cell Extraction Buffer SSW 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 1X Cell Extraction Buffer SSW 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 1:4 into 1X Cell Extraction Buffer SSW 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 4BI 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

Copyright © 2024 Abcam, All Rights Reserved. All information / detail is correct at time of going to print.
Version 1a | 2024-10-25

ab322084 – Gentamicin SimpleStep ELISA® Kit – Extracellular

Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of Gentamicin.

The standard in this kit is free gentamicin.

Spiked experiments were used to validate serum, 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.

This kit is incompatible with plasma (heparin).

For the measurement of Gentamicin in cell and tissue extracts use Gentamicin ELISA Kit - Intracellular ab322085.

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 Gentamicin.

Compound	Cross Reactivity – 4,000 pg/mL (%)	Cross Reactivity – 40,000 pg/mL (%)
Gentamicin	100	-
Geneticin (G418)	100	-
Kanamycin	0	0
Neomycin	0	0
Streptomycin	0	0

INTERFERENCE

4,000 and 40,000 pg/mL of Kanamycin, Neomycin, and Streptomycin were tested for interference with 500 pg/mL of Gentamicin. 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	0.030	0.032	0.031	-
0 (B ₀)	1.647	1.690	1.669	100%
62.5	1.456	1.382	1.419	85%
125	1.161	1.077	1.119	67%
250	0.774	0.791	0.783	47%
500	0.490	0.484	0.487	29%
1,000	0.277	0.274	0.275	17%
2,000	0.137	0.144	0.140	8%

Table 1. Example of species Gentamicin standard curve in 1X Cell Extraction Buffer SSW. The Gentamicin 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 55.11 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 Gentamicin 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 (%)
10% Human Serum	116	112 - 118
10% Human Plasma – Citrate	113	109 - 115
10% Human Plasma – EDTA	108	107 - 110
10% Human Urine	87	86 - 90
10% Human Breast Milk	99	97 - 100
10% Mouse Serum	112	110 - 114
10% Mouse Plasma – Citrate	108	105 - 110
10% Mouse Plasma – EDTA	109	107 - 110
25% Cell Culture Media*	107	105 - 110

*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.

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

Dilution Factor	Interpolated value	10% Human Serum	10% Human Plasma (Citrate)	10% Human Plasma (EDTA)
Undiluted	pg/mL	1,191	1,127	1,155
	% Expected value	100	100	100
2	pg/mL	545	544	514
	% Expected value	92	97	89
4	pg/mL	281	268	247
	% Expected value	94	95	86
8	pg/mL	139	114	125
	% Expected value	94	81	87

Dilution Factor	Interpolated value	10% Mouse Serum	10% Mouse Plasma (Citrate)	10% Mouse Plasma (EDTA)
Undiluted	pg/mL	1,104	1,080	1,050
	% Expected value	100	100	100
2	pg/mL	479	508	469
	% Expected value	87	94	89
4	pg/mL	260	265	249
	% Expected value	94	98	95
8	pg/mL	139	133	127
	% Expected value	101	98	97

Dilution Factor	Interpolated value	10% Human Urine	10% Human Milk	25% Media*
Undiluted	pg/mL	853	927	547
	% Expected value	100	100	100
2	pg/mL	471	504	262
	% Expected value	110	109	96
4	pg/mL	219	241	139
	% Expected value	103	104	101
8	pg/mL	107	119	68
	% Expected value	100	103	99

*Media is DMEM containing 10% fetal calf serum

Precision

Mean coefficient of variations of interpolated values of Gentamicin from 10% Human Serum spiked with Gentamicin within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	5.8	6.6

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