TECHNICAL DATA SHEET

AlphaLISA® Research Reagents

AlphaLISA Chloramphenicol Biotin-Free Detection Kit

Product number: AL393 HV/C/F

Lot number: sample

Manufacturing date:

Caution: For Laboratory Use. A research product for research purposes only.

Contents

Product Information

Application: This kit is designed for the quantitative determination of Chloramphenicol in buffered solution, milk and cell culture supernatants using a homogeneous AlphaLISA assay (no wash steps). The kit utilizes a Digoxigenin (DIG) / Anti-DIG interaction as opposed to the traditional Streptavidin/Biotin interaction. This enables optimal performance when working with biotin-rich media (e.g. RPMI) or samples containing endogenous biotin.

Sensitivity: Lower Detection Limit (LDL): 37.5 pg/mL

Lower Limit of Quantification (LLOQ): 733.6 pg/mL

EC_{50}: 11.9 ng/mL

Dynamic range: 0.038 – 1 000 000 ng/mL (Figure 1).

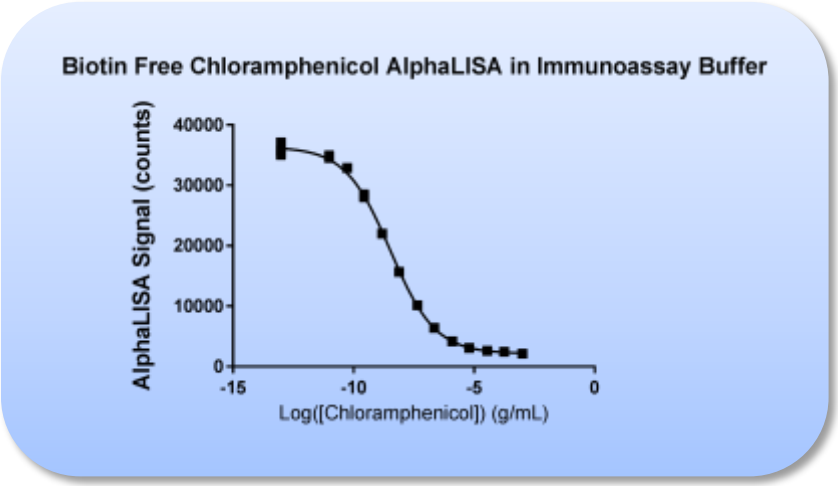


Figure1. Typical sensitivity curves in AlphaLISA Immunoassay Buffer. The data was generated using a white Optiplate™ 384 microplate and the EnVision® Multilabel Plate Reader 2103 with Alpha option.

Storage: Store kit in the dark at +4˚C. Store analyte at 4°C for up to 6 weeks. Aliquot and store tracer at -20 °C for up to 6 weeks.

Stability: This kit is stable for at least 6 months from the manufacturing date when stored in its original packaging and the recommended storage conditions.

Quality Control

Lot to lot consistency is confirmed in an AlphaLISA assay. Maximum and minimum signals, EC_{50} and LDL were measured on the EnVision Multilabel Plate Reader with Alpha option using the protocol described in this technical data sheet. We certify that these results meet our quality release criteria. Maximum counts may vary between bead lots and the instrument used, with no impact on LDL measurement.

EC_{50}:	4.830 ng/mL
LDL:	70.10 pg/mL
LLOQ:	1112 pg/mL
Min Counts:	1404
Max Counts:	66384

Analyte of Interest

Chloramphenicol is a broad spectrum antibiotic derived from the bacterium, *Streptomyces venezuelae,* that can be used to treat infections such as meningitis, plague, cholera, and typhoid fever. Due to its severe side effects, blood levels during treatment are monitored closely. The use of chloramphenicol to treat food-producing animals is banned in the United States, Canada, EU, and Australia due to the high potential risk of severe side effects. Food and water sources are strictly monitored for residues as any supply that is contaminated with chloramphenicol producing bacteria can result in exposure to humans.

Description of the AlphaLISA Assay

AlphaLISA technology allows the detection of molecules of interest in buffer, cell culture media, serum and plasma in a highly sensitive, quantitative, reproducible and user-friendly mode. In this AlphaLISA assay, a DIG-labeled Anti-Analyte Antibody binds to the anti-DIG Alpha Donor beads, while another Anti-Analyte Antibody is conjugated to AlphaLISA Acceptor beads. In the presence of the analyte, the beads come into close proximity. The excitation of the Donor beads provokes the release of singlet oxygen molecules that triggers a cascade of energy transfer in the Acceptor beads, resulting in a sharp peak of light emission at 615 nm (Figure 2).

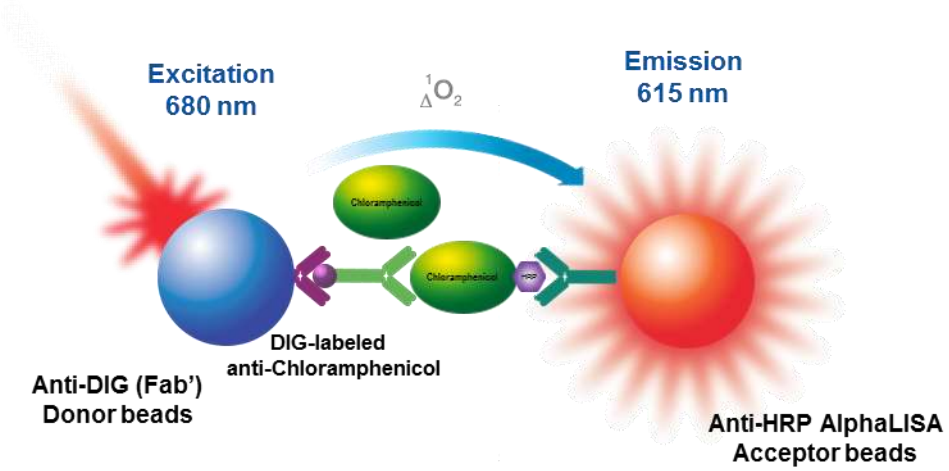


Figure 2. AlphaLISA Assay Principle.

Precautions

- The Alpha Donor beads are light-sensitive. All the other assay reagents can be used under normal light conditions. All Alpha assays using the Donor beads should be performed under subdued laboratory lighting (< 100 lux). Green filters (LEE 090 filters (preferred) or Roscolux filters #389 from Rosco) can be applied to light fixtures.
- All blood components and biological materials should be handled as potentially hazardous.
- Some analytes are present in blood. Take precautionary measures to avoid contamination of the reagent solutions.
- The DIG Labeled Anti-Analyte Antibody contains sodium azide. Contact with skin or inhalation should be avoided.

Kit Contents

Kit components	AL393HV (100 assay points***)	AL393C (500 assay points***)	AL393F (5000 assay points***)
AlphaLISA Anti-HRP Acceptor beads stored in PBS, 0.05% Proclin-300, pH 7.2	40 µL @ 5 mg/mL (1 brown tube, white cap)	100 µL @ 5 mg/mL (1 brown tube, white cap)	1 mL @ 5 mg/mL (1 brown tube, white cap)
Anti-Digoxigenin Fab Fragment Donor beads stored in 25 mM HEPES, 100 mM NaCl, 0.05% Proclin-300, pH 7.4	80 µL @ 5 mg/mL (1 brown tube, black cap)	200 µL @ 5 mg/mL (1 brown tube, black cap)	2 x 1 mL @ 5 mg/mL (2 brown tubes, black caps)
DIG Labeled Anti-Chloramphenicol Antibody stored in PBS, 0.1% Tween-20, 0.05% NaN_3, pH 7.4	20 µL @ 500 nM (1 tube, black cap)	50 µL @ 500 nM (1 tube, black cap)	500 µL µL @ 500 nM (1 tube, black cap)
Chloramphenicol Analyte* Ethanol/Isopropanol Solution	20 µL @ 100 mg/mL (1 tube, clear cap)	20 µL @ 100 mg/mL (1 tube, clear cap)	20 µL @ 100 mg/mL (1 tube, clear cap)
Chloramphenicol-HRP Tracer	20 µL @ 500X (1 tube, green cap)	50 µL @ 500X (1 tube, green cap)	500 µL µL @ 500X (1 tube, green cap)
AlphaLISA Immunoassay Buffer (10X) **	2 mL, 1 small bottle	10 mL, 1 small bottle	100 mL, 1 large bottle

* It has been demonstrated that reconstituted Chloramphenicol is stable for at least 1 year at 4°C. One vial contains an amount of Chloramphenicol sufficient for performing 10 standard curves.

** Contains 250 mM HEPES, pH 7.4, 1% Casein, 10 mg/mL Dextran-500, 5% Triton X-100 and 0.5% Proclin-300. Extra buffer can be ordered separately (cat # AL000C: 10 mL, cat # AL000F: 100 mL).
Note: 10X buffer might be slightly yellow. However, this does not affect the assay results.

*** The number of assay points is based on an assay volume of 100 µL in 96-well plates or 50 µL in 384-well assay plates using the kit components at the recommended concentrations.

Sodium azide should **not** be added to the stock reagents. High concentrations of sodium azide (> 0.001 % final in the assay) might decrease the AlphaLISA signal.

Specific additional required reagents and materials:

The following materials are recommended:

Item	Suggested source	Catalog #
TopSeal™-A Plus Adhesive Sealing Film	PerkinElmer Inc.	6050185
EnVision®-Alpha Reader	PerkinElmer Inc.	-

Recommendations

- The volume indicated on each tube is guaranteed for single pipetting. Multiple pipetting of the reagents may reduce

the theoretical amount left in the tube. To minimize loss when pipetting beads, it is preferable not to pre-wet the tip.

- Centrifuge all tubes (including lyophilized analyte) before use to improve recovery of content (2000g, 10-15 sec). Re-suspend all reagents by vortexing before use.
- Use Milli-Q® grade H_2O (18 MΩ•cm) to dilute 10X AlphaLISA Immunoassay Buffer and to reconstitute the lyophilized analyte.
- When diluting the standard or samples, change tips between each standard or sample dilution. When loading reagents in the assay microplate, change tips between each standard or sample addition and after each set of reagents.
- When reagents are added to the microplate, make sure the liquids are at the bottom of the well.
- Small volumes may be prone to evaporation. It is recommended to cover microplates with TopSeal-A Plus Adhesive Sealing Films to reduce evaporation during incubation. Microplates can be read with the TopSeal-A Plus Film.
- The AlphaLISA signal is detected with an EnVision Multilabel Reader equipped with the Alpha option using the AlphaScreen standard settings (e.g. Total Measurement Time: 550 ms, Laser 680 nm Excitation Time: 180 ms, Mirror: D640as, Emission Filter: M570w, Center Wavelength 570 nm, Bandwidth 100 nm, Transmittance 75%).
- AlphaLISA signal will vary with temperature and incubation time. For consistent results, identical incubation times and temperature should be used for each plate.
- The standard curves shown in this technical data sheet are provided for information only. A standard curve must be generated for each experiment. The standard curve should be performed in the fetal bovine serum for serum and/or plasma samples.

Assay Procedure

IMPORTANT: PLEASE READ THE RECOMMENDATIONS BELOW BEFORE USE

- The protocol described below is an example for generating one standard curve in a 50 µL final assay volume (48 wells, triplicate determinations). The protocols also include testing samples in 452 wells. If a different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly, as shown in the table below. These calculations do not include excess reagent to account for losses during transfer of solutions or dead volumes.
- The standard dilution protocol is provided for information only. As needed, the number of replicates or the range of concentrations covered can be modified.
- Use of four background points in triplicate (12 wells) is recommended when LDL/LLOQ is calculated. One background point in triplicate (3 wells) can be used when LDL/LLOQ is not calculated.

		Volume					
Format	**# of data points**	**Final**	**Sample**	**AlphaLISA beads/ Tracer MIX**	**DIG labeled Antibody**	**Donor beads**	**Plate recommendation**
AL393HV	100	100 µL	10 µL	20 µL	20 µL	50 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
AL393C	250	100 µL	10 µL	20 µL	20 µL	50 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
	500	50 µL	5 µL	10 µL	10 µL	25 µL	White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate™-384 (cat # 6005350)
	1 250	20 µL	2 µL	4 µL	4 µL	10 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate™-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	2 500	10 µL	1 µL	2 µL	2 µL	5 µL	Light gray AlphaPlate-1536 (cat # 6004350)
AL393F	5 000	50 µL	5 µL	10 µL	10 µL	25 µL	White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate-384 (cat # 6005350)
	12 500	20 µL	2 µL	4 µL	4 µL	10 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	25 000	10 µL	1 µL	2 µL	2 µL	5 µL	Light gray AlphaPlate-1536 (cat # 6004350)

The protocol (3 incubation steps) described below is for 500 assay points including one standard curve (48 wells) and samples (452 wells).
If a different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly.

1) Preparation of 1X AlphaLISA Immunoassay Buffer:

Add 5 mL of 10X AlphaLISA Immunoassay Buffer to 45 mL H_2O.

2) Preparation of 5X MIX AlphaLISA Anti-HRP Acceptor beads (100 µg/mL) + Chloramphenicol-HRP Tracer (5X):
   a. Add 100 µL of 5 mg/mL AlphaLISA Anti-HRP Acceptor beads and 50 µL of 500X Chloramphenicol-HRP Tracer to 4850 µL of 1X AlphaLISA Immunoassay Buffer.

Incubate for overnight at 4 °C. This is to pre-bind HRP-tracer to the Acceptor beads.

3) Preparation of Chloramphenicol analyte standard dilutions:

   a. Chloramphenicol analyte is provided at 100 mg/mL in ethanol/isopropanol solution, use directly.
   b. Prepare standard dilutions as follows in 1X AlphaLISA Immunoassay Buffer (change tip between each standard dilution):

Tube	Vol. of Chloramphenicol (µL)	Vol. of diluent (µL) *	[Chloramphenicol] in standard curve	
			(g/mL in 5 µL)	(ng/mL in 5 µL)
A	2 µL of Chloramphenicol Solution	198	1.00E-03	1 000 000
B	30 µL of tube A	140	1.76E-04	176 000
C	30 µL of tube B	120	3.53E-05	35 300
D	30 µL of tube C	140	6.23E-06	6 230
E	30 µL of tube D	120	1.25E-06	1 250
F	30 µL of tube E	140	2.20E-07	220
G	30 µL of tube F	120	4.40E-08	44.0
H	30 µL of tube G	140	7.76E-09	7.76
I	30 µL of tube H	120	1.55E-09	1.55
J	30 µL of tube I	140	2.74E-10	0.27
K	30 µL of tube J	120	5.48E-11	0.055
L	30 µL of tube K	140	9.66E-12	0.0097
M ** (background)	0	100	0	0
N ** (background)	0	100	0	0
O ** (background)	0	100	0	0
P ** (background)	0	100	0	0

* Dilute standards in diluent (e.g. 1X AlphaLISA Immunoassay Buffer).
At low concentrations of analyte, a significant amount of analyte can bind to the vial. Therefore, load the analyte standard dilutions in the assay microplate within 60 minutes of preparation.

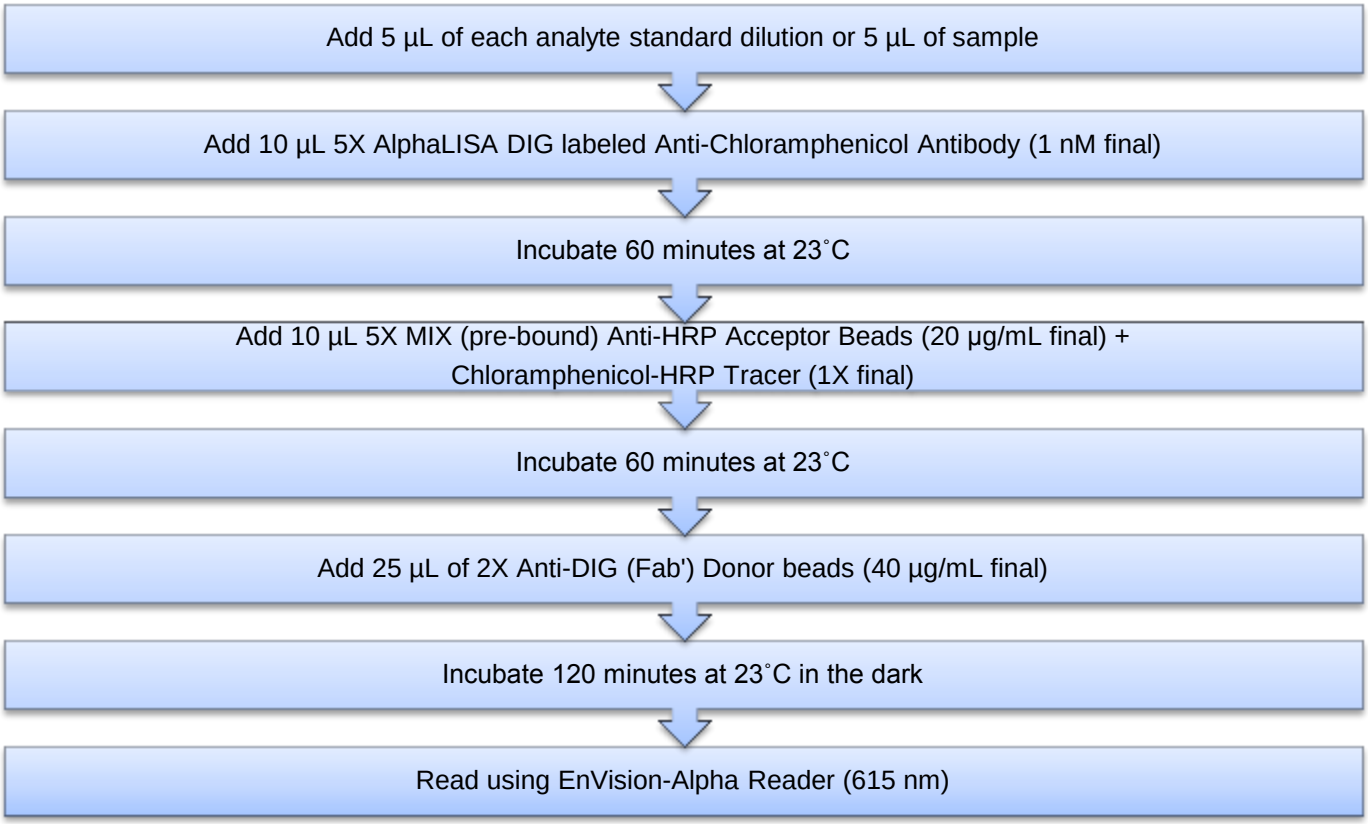
** Four background points in triplicate (12 wells) are used when LDL is calculated. If LDL does not need to be calculated, one background point in triplicate can be used (3 wells).

4) Preparation of 5X DIG labeled Anti-Chloramphenicol antibody (5 nM):
   a. Prepare just before use.
   b. Add 50 µL of 500 nM DIG labeled Anti-Chloramphenicol antibody to 4950 µL of 1X AlphaLISA Immunoassay Buffer.

5) Preparation of 2X Anti-DIG (Fab') Donor beads (80 µg/mL): Keep the beads under subdued laboratory lighting.

a. Prepare just before use
b. Add 200 µL of 5 mg/mL Anti-DIG (Fab')-Donor beads to 12 300 µL of 1X AlphaLISA Immunoassay Buffer.

6) In a white Optiplate (384 wells):



Data Analysis

- Calculate the average count value for the background wells.
- Generate a standard curve by plotting the AlphaLISA counts versus the concentration of analyte. A log scale can be used for either or both axes. No additional data transformation is required.
- Analyze data according to a nonlinear regression using the 4-parameter logistic equation (sigmoidal dose-response curve with variable slope) and a $1/Y^2$ data weighting (the values at maximal concentrations of analyte after the hook point should be removed for correct analysis).
- The LDL is calculated by interpolating the average background counts (12 wells without analyte) + 3 x standard deviation value (average background counts + (3xSD)) on the standard curve.
- The LLOQ as measured here is calculated by interpolating the average background counts (12 wells without analyte) + 10 x standard deviation value (average background counts + (10xSD)) on the standard curve. Alternatively, the true LLOQ can be determined by spiking known concentrations of analyte in the matrix and measuring the percent recovery, and then determining the minimal amount of spiked analyte that can be quantified within a given limit (usually +/- 20% or 30% of the real concentration).
- Read from the standard curve the concentration of analyte contained in the samples.
- If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Assay Performance Characteristics

AlphaLISA assay performance described below was determined using the 3 step protocol performed in AlphaLISA Immunoassay Buffer (IAB), Nonfat Milk, and RPMI + 10% FBS.

- Assay Sensitivity:

The LDL was calculated as described above. The values correspond to the lowest concentration of analyte that can be detected in a volume of 5 µL using the recommended assay conditions.

LDL (pg/mL)	Buffer/Media	# of experiments
37.5	IAB	9
38.5	Nonfat Milk	6
57.8	RPMI with 10% FBS	6

*Note that LDL can be decreased (i.e. sensitivity increased) by increasing the volume of analyte in the assay (e.g. use 10 µL of analyte in a final assay volume of 50 µL).

- Assay Precision:

The following assay precision data were calculated from the three independent assays using two different kit lots. For each lot, the standard curves were prepared in IAB, Nonfat Milk, or RPMI supplemented with 10% FBS. Each assay consisted of one standard curve comprising 12 data points (each in triplicate) and 12 background wells (no analytes). The assays were performed in 384-well format using IAB.

- Intra-assay precision:

The intra-assay precision was determined using a total of 7 independent determinations in triplicate, shown as CV%.

Chloramphenicol	IAB	Nonfat Milk	RPMI with 10% FBS
CV%	4	5	5

- Inter-assay precision:

The inter-assay precision was determined using a total of 7 independent determinations with 21 measurements. Shown as CV%.

Chloramphenicol	IAB	Nonfat Milk	RPMI with 10% FBS
CV%	6	8	8

- Spike Recovery:

Three known concentrations of analyte were spiked into IAB and cell culture media supplemented with 10% FBS. The average recovery from three independent measurements is reported.

Spiked Chloramphenicol (ng/mL)	% Recovery		
	IAB	Nonfat Milk	RPMI with 10% FBS
200	89	93	91
40	95	94	90
5	106	101	98

- Specificity:

Cross-reactivity of the Chloramphenicol AlphaLISA Kit was tested using the following analogues at 40 ng/mL in AlphaLISA Immunoassay Buffer.

Protein	% Cross-reactivity
Thiamphenicol	< 0.1 %
Florfenicol	< 0.1%

Troubleshooting Guide

You will find detailed recommendations for common situations you might encounter with your AlphaLISA Assay kit at:

http://www.perkinelmer.com/resources/technicalresources/applicationsupportknowledgebase/alphalisa-alphascreen-no-washassays/alpha_troubleshoot.xhtml

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

This product is not for resale or distribution except by authorized distributors.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com