



AlphaLISA[®] HCVcAg Detection Kit

Product number: AL3092 HV/C/F

Research Use Only. Not for use in diagnostic procedures.

Product Information

Application: This kit is designed for the quantitative determination of HCV cAg in cell culture media using a homogeneous AlphaLISA assay (no wash steps).

Sensitivity: Lower Detection Limit (LDL): 374 pg/mL
Lower Limit of Quantification (LLOQ): 1190 pg/mL
EC₅₀: 259 ng/mL

Dynamic range: 374 – 1 000 000 pg/mL

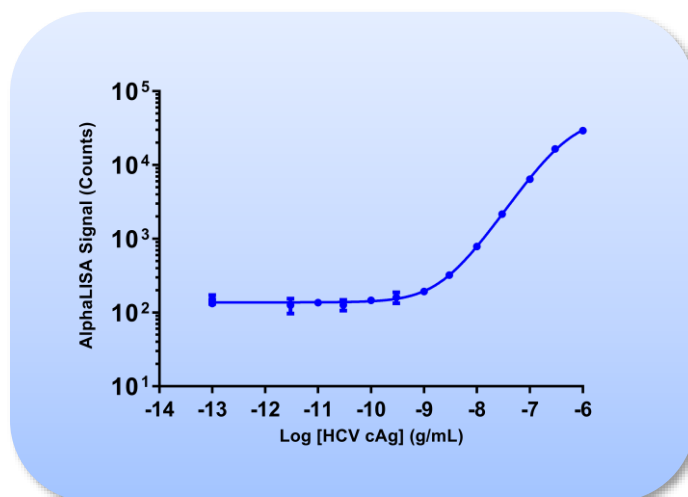


Figure 1. Typical sensitivity curve in AlphaLISA Immunoassay Buffer. The data was generated using a white Optiplate[™]-384 microplate and the EnVision[®] Multilabel Plate Reader 2102 with Alpha option.

Storage: Store kit, except analyte, in the dark at +4°C. Store the analyte and diluted analyte aliquots at -20 °C. Avoid freeze-thaw cycles.

Stability: This kit is stable for at least 12 months from the manufacturing date when stored in its original packaging and the recommended storage conditions. Undiluted analyte stored at -20 °C is stable for at least 3 months from the date of manufacturing.

Analyte of Interest

The hepatitis C virus is one of many viruses that causes liver diseases (liver inflammation and liver fibrosis) and it is transmitted via blood such as unprotected sexual activity, blood transfusion, mother-to-infant transmission, or occupational exposure to blood. About 143 (2%) million people worldwide have been infected with hepatitis C. Chronic HCV infection often leads to liver cirrhosis and failure. The infection can be detected by HCV serology and detection of HCV antibodies. Quantitative detection of HCV cAg has been used increasingly as the most important marker for monitoring HCV titer, disease progression, and assessing antiviral treatment.

Description of the AlphaLISA Assay

AlphaLISA technology allows the detection of molecules of interest in buffer and cell culture media in a highly sensitive, quantitative, reproducible and user-friendly mode. In this AlphaLISA assay, a Biotinylated Anti-HCV cAg Antibody binds to the Streptavidin-coated Alpha Donor beads, while another Anti-HCV cAg Antibody is conjugated to AlphaLISA Acceptor beads. In the presence of the HCV cAg, 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).

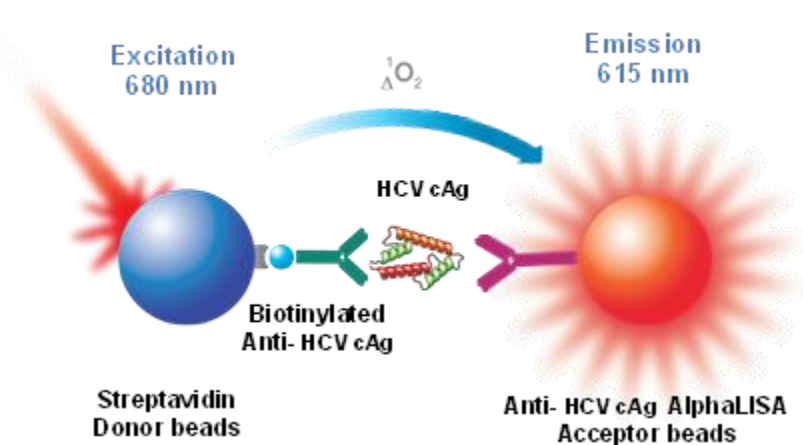


Figure 2. AlphaLISA HCV cAg Assay Principle.

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.
- Take precautionary measures to avoid contamination of the reagent solutions.
- The Biotinylated Anti-Analyte Antibody contains sodium azide. Contact with skin or inhalation should be avoided.

Kit Content: Reagents and Materials

Kit components	AL3092HV (100 assay points ^{***})	AL3092C (500 assay points ^{***})	AL3092F (5000 assay points ^{***})
AlphaLISA Anti-HCV cAg Acceptor beads stored in PBS, 0.05% Kathon, pH 7.2	20 µL @ 5 mg/mL (1 brown tube, <u>white</u> cap)	50 µL @ 5 mg/mL (1 brown tube, <u>white</u> cap)	500 µL @ 5 mg/mL (1 brown tube, <u>white</u> cap)
Streptavidin (SA)-coated Donor beads stored in 25 mM HEPES, 100 mM NaCl, 0.05% Kathon, pH 7.4	40 µL @ 5 mg/mL (1 brown tube, <u>black</u> cap)	100 µL @ 5 mg/mL (1 brown tube, <u>black</u> cap)	1 mL @ 5 mg/mL (1 brown tube, <u>black</u> cap)
Biotinylated Anti-HCV cAg Antibody stored in PBS, 0.1% Tween-20, 0.05% NaN ₃ , pH 7.4	20 µL @ 500 nM (1 tube, <u>black</u> cap)	50 µL @ 500 nM (1 tube, <u>black</u> cap)	500 µL @ 500 nM (1 tube, <u>black</u> cap)
HCV cAg Analyte stored in IAB	20 µL @ 100 µg/mL (1 tube, <u>clear</u> cap)	20 µL @ 100 µg/mL (1 tube, <u>clear</u> cap)	20 µL @ 100 µg/mL (1 tube, <u>clear</u> cap)
AlphaLISA Immunoassay Buffer (10X)	2 mL, 1 small bottle	10 mL, 1 medium bottle	100 mL, 1 large bottle

* Dilute analyte with 1X AlphaLISA Immunoassay Buffer (see page 7 for more details). The diluted analyte should be used within 60 minutes or aliquoted into screw-capped 0.5 mL polypropylene vials and stored at -20°C for future experiments. The aliquoted analyte at -20°C is stable up to 84 days. Avoid freeze-thaw cycles. One vial contains an amount of analyte sufficient for performing 20 standard curves. Additional vials can be ordered separately (cat # AL3092S).

** Extra buffer can be ordered separately (cat # AL000C: 10 mL, cat # AL000F: 100 mL).

*** The number of assay points is based on an assay volume of 100 µL in 96-well plates or 50 µL in 96- or 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. Note that sodium azide from the Biotinylated Antibody stock solution will not interfere with the AlphaLISA signal (0.0001% final in the assay).

Specific additional required reagents and materials:

The following materials are recommended:

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

Recommendations

IMPORTANT: PLEASE READ THE RECOMMENDATIONS BELOW BEFORE USE

- 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 H₂O (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 Adhesive Sealing Films to reduce evaporation during incubation. Microplates can be read with the TopSeal-A Film in place.
- The AlphaLISA signal is detected with an EnVision Multilabel Plate 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.

Assay Procedure

- The manual described below is an example for generating one standard curve in a 50 µL final assay volume (48 wells, triplicate determinations). The manuals also include testing samples in 452 wells. If 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 manual 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.

Format	# of data points	Volume					Plate recommendation
		Final	Sample	AlphaLISA Acceptor Beads	Biotinylated Antibody	SA-Donor beads	
AL3092HV	100	100 µL	10 µL	20 µL	20 µL	50 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
AL3092C	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)
AL3092F	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)

Manual for HCVcAg AlphaLISA Assay

3 Step Manual (3 incubation steps) – Dilution of standards can be done in 1X AlphaLISA Immunoassay Buffer. The manual described below is for one standard curve (48 wells). *If a different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly.*

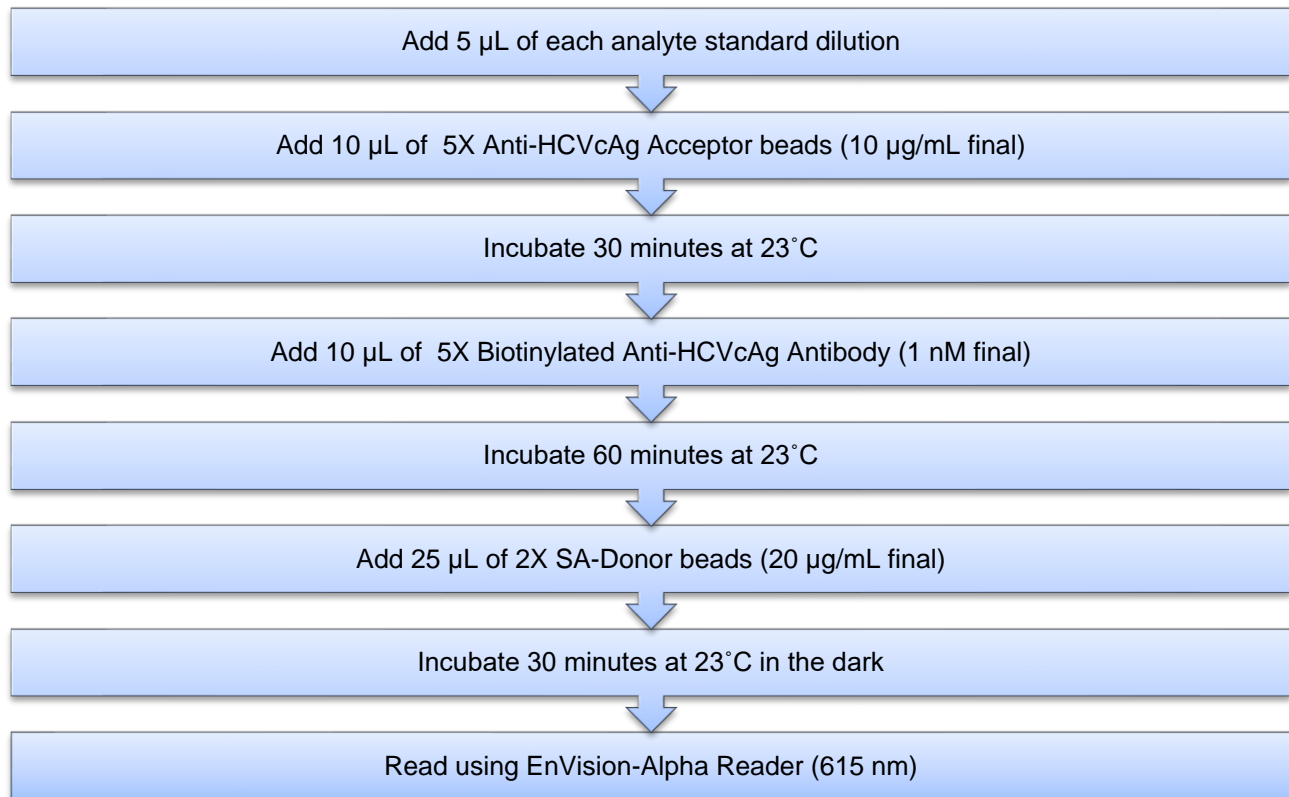
- 1) Preparation of 1X AlphaLISA Immunoassay Buffer:
Add 1 mL of 10X AlphaLISA Immunoassay Buffer to 9 mL Milli-Q® grade H₂O.
- 2) Preparation of HCVcAg analyte standard dilutions:
 - a. Thaw 20 µL sample of HCVcAg.
 - b. Add 180 µL of 1X AlphaLISA Immunoassay Buffer to 20 µL (2 µg) HCVcAg.
 - c. Aliquot and store unused diluted analyte standard at -20 °C.
 - d. Prepare standard dilutions as follows in 1X AlphaLISA Immunoassay Buffer (change tip between each standard dilution):

Tube	Vol. of HCVcAg (µL)	Vol. of diluent (µL) *	[HCVcAg] in standard curve	
			(g/mL in 5 µL)	(pg/mL in 5 µL)
A	10 µL of diluted HCVcAg	90	1.0E-06	1 000 000
B	60 µL of tube A	140	3.0E-07	300 000
C	60 µL of tube B	120	1.0E-07	100 000
D	60 µL of tube C	140	3.0E-08	30 000
E	60 µL of tube D	120	1.0E-08	10 000
F	60 µL of tube E	140	3.0E-09	3 000
G	60 µL of tube F	120	1.0E-09	1 000
H	60 µL of tube G	140	3.0E-10	300
I	60 µL of tube H	120	1.0E-10	100
J	60 µL of tube I	140	3.0E-11	30
K	60 µL of tube J	120	1.0E-11	10
L	60 µL of tube K	140	3.0E-12	3
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).

- 3) Preparation of 5X AlphaLISA Anti-HCVcAg Antibody Acceptor beads (50 µg/mL):
 - a. Prepare just before use.
 - b. Add 50 µL of 5 mg/mL AlphaLISA Anti- HCVcAg Antibody Acceptor to 4950 µL of 1X AlphaLISA Immunoassay Buffer.
- 4) Preparation of 5X Biotinylated Anti- HCVcAg Antibody (5 nM):
 - a. Prepare just before use.
 - b. Add 50 µL of 500 nM Biotinylated Anti- HCVcAg Antibody to 4950 µL of 1X AlphaLISA Immunoassay Buffer.
- 5) Preparation of 2X Streptavidin (SA) Donor beads (40 µg/mL):
 - a. Prepare just before use.
 - b. Keep the beads under subdued laboratory lighting.
 - c. Add 100 µL of 5 mg/mL SA-Donor beads to 12 400 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 manual using AlphaLISA Immunoassay Buffer (IAB) as assay buffer. The analytes (standards) were prepared in IAB, DMEM + 10% FBS, or RPMI + 10% FBS, and all other components were prepared in IAB.

- 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 μ L sample using the recommended assay conditions.

LDL (pg/mL)*	(Analyte diluent)	# of experiments
374	IAB	9
1042	DMEM + 10% FBS	6
11946	RPMI + 10% FBS	1

* Note that LDL can be decreased (i.e. sensitivity increased) by increasing the volume of analyte in the assay (e.g. use 10 μ L of analyte in a final assay volume of 50 μ L). **Use of RPMI with 10%FBS should be avoided.**

- Assay Precision:

The following assay precision data were calculated from the three independent assays using two different kit lots. In each lot, the analytes were prepared in IAB and DMEM + 10% FBS. All other components were prepared in IAB. 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.

- Intra-assay precision:

The intra-assay precision was determined using a total of 16 independent determinations in triplicate. Shown as CV%.

HCV cAg	IAB	DMEM + 10% FBS
CV (%)	7	8

- Inter-assay precision:

The inter-assay precision was determined using a total of 3 independent determinations with 9 measurements for 3 ng/mL sample. Shown as CV%.

HCV cAg	IAB	DMEM + 10% FBS
CV (%)	7	15

- Spike Recovery:

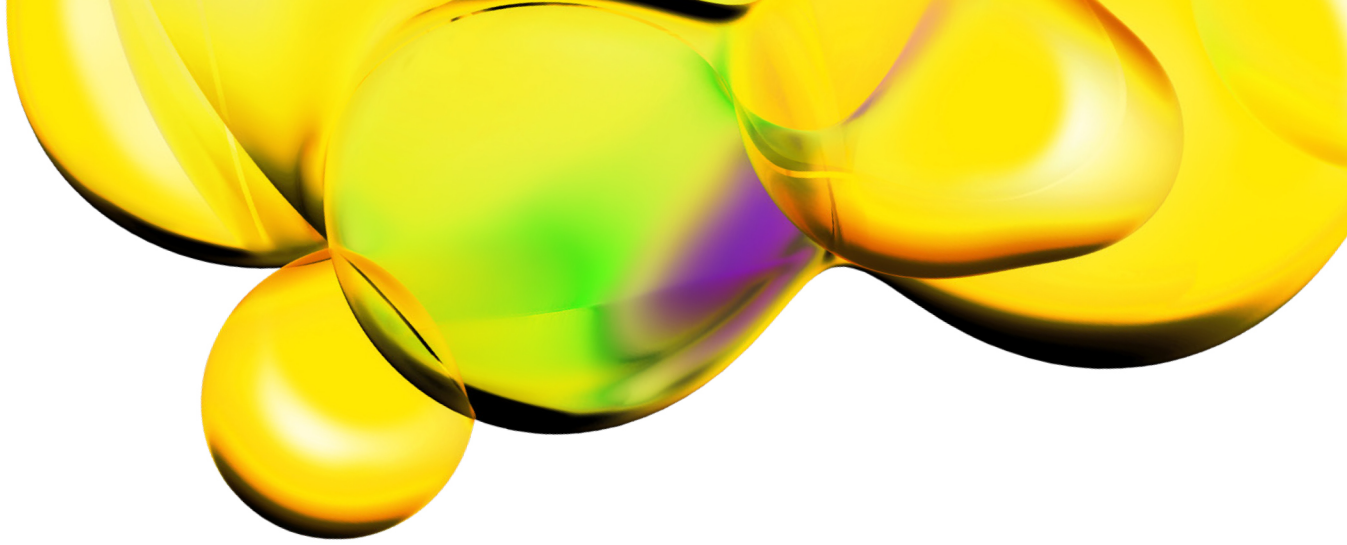
Three known concentrations of analyte were spiked into IAB and DMEM + 10% FBS. All samples, including non-spiked diluents were measured in the assay. Note that the analytes for the respective standard curves were prepared in IAB and DMEM + 10% FBS. All other assay components were diluted in IAB.

Spiked HCV cAg (ng/mL)	% Recovery	
	IAB	DMEM + 10% FBS
30	95	94
10	87	95
3	86	96

Troubleshooting Guide

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

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