MANUAL

AlphaLISA® Host Cell Residual DNA Detection Kit

Product number: AL331 HV/C/F

Research Use Only. Not for use in diagnostic procedures.

Product Information

Application: This kit is designed for the quantitative determination of DNA contamination in cell culture supernatants using a homogeneous AlphaLISA assay (no wash steps). This kit can be used with DNA from different prokaryotic and eukaryotic species.

Sensitivity: Lower Detection Limit (LDL): 0.06 ng/mL

Lower Limit of Quantification (LLOQ): 0.22 ng/mL

EC_{50}: 46.8 ng/mL

Dynamic range: 0.06- 100 ng/mL (Figure 1).

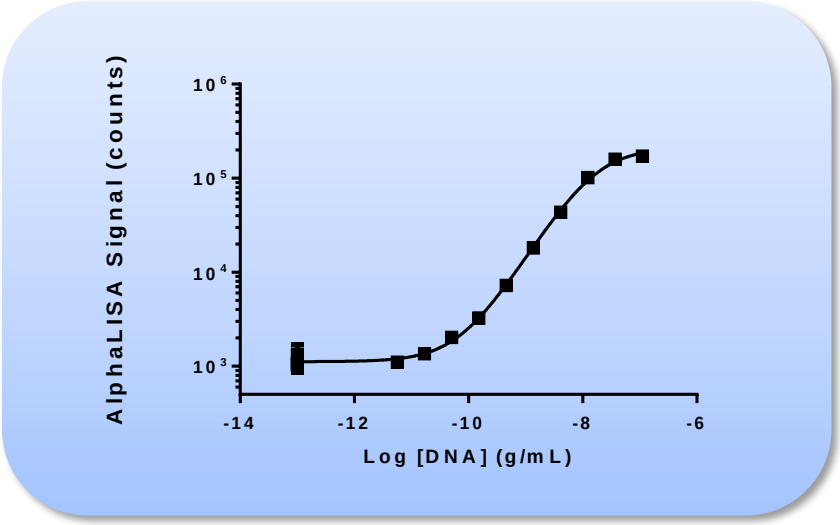


Figure 1. Typical sensitivity curves in AlphaLISA DNA Detection Buffer. The data was generated using a white CulturPlate-384 microplate and the EnVision® 2103 Multilabel Plate Reader with Alpha option.

Storage: Store kit in the dark at +4˚C. Store reconstituted analyte at -20°C.

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. Note: The DNA analyte is stable for at least 18 months when stored at -20°C.

Analyte of Interest

Biologics can be impacted negatively by contamination with DNA introduced during fermentation and purification processes. While the use of serum-free media in the manufacturing process significantly improved the success rate on preventing DNA impurities, other routes of contamination, such as microbial contamination, still remain a concern. It is thus critical to remove and monitor DNA impurities at each step in the purification process. This kit is designed to quantify the levels of DNA (from different hosts, either double- or single-stranded and of varying fragment sizes) in cell culture supernatants.

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 an AlphaLISA assay, a Biotinylated Anti-Analyte Antibody binds to the Streptavidin-coated 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).

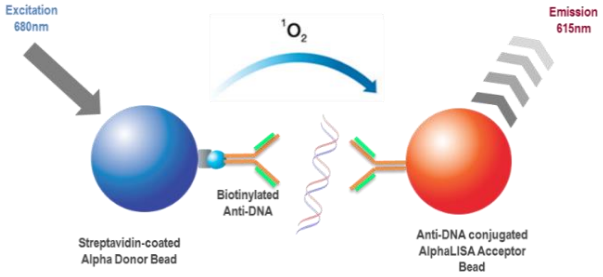


Figure 2. AlphaLISA Assay principle.

Precautions

- The AlphaScreen® 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. The analyte included in this kit is from a human source.
- Some analytes are present in saliva. 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	AL331HV (100 assay points**)	AL331C (500 assay points**)	AL331F (5 000 assay points**)
AlphaLISA Anti-DNA Acceptor beads stored in PBS, 0.05% Kathon, pH 7.2	20 µL @ 5 mg/mL (1 brown tube, white cap)	50 µL @ 5 mg/mL (1 brown tube, white cap)	500 µL @ 5 mg/mL (1 brown tube, white cap)
SA Donor beads stored in 25 mM HEPES, 100 mM NaCl, 0.05% Kathon, pH 7.4	20 µL @ 5 mg/mL (1 brown tube, black cap)	50 µL @ 5 mg/mL (1 brown tube, black cap)	500 µL @ 5 mg/mL (1 brown tube, black cap)
Biotinylated antibody stored in PBS, 0.1% Tween-20, 0.05% NaN3, pH 7.4	20 µL @ 500 nM (1 tube, black cap)	50 µL @ 500 nM (1 tube, black cap)	500 µL @ 500 nM (1 tube, black cap)
DNA analyte standard (in Tris EDTA buffer)	100 µL @ 10 µg/mL 1 tube, clear cap	100 µL @ 10 µg/mL 1 tube, clear cap	100 µL @ 10 µg/mL 1 tube, clear cap
AlphaLISA DNA Detection Buffer (10X)	2 mL, 1 small bottle	10 mL, 1 small bottle	100 mL, 1 large bottle

* Analyte should be used within 60 minutes or aliquoted into screw-capped polypropylene vials and stored at -20°C for further experiments. Avoid multiple freeze-thaw cycles. It has been demonstrated that reconstituted it is stable for at least 18 months at -20°C. One vial contains an amount of DNA sufficient for performing 10 standard curves. Additional vials can be ordered separately (cat # AL331S).

** 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:

Item	Suggested source	Catalog #
UltraPure BSA (50 mg/ml)	Life Technologies	AM2618
Water, Sequencing Grade	VWR	K683-4L

The following materials are recommended:

Item	Suggested source	Catalog #
TopSeal™-A Adhesive Sealing Film	Revvity Inc.	6050195
EnVision®-Alpha Reader	Revvity Inc.	-
White sterile CulturPlates	Revvity Inc.	6005680 6007680 6004680

Recommendations

General 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 before use to improve recovery of content (2000g, 10-15 sec).
- Use Sequencing Grade Water (18 MΩ•cm) to dilute 10X DNA Detection Buffer
- 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.
- 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 DNA Detection Buffer for serum and/or plasma samples.

Specific recommendations:

This assay is extremely sensitive to DNA contamination and nucleases which can be introduced at any stage and could cause inconsistent results. Since DNA fragments can be found on most surfaces, please take every precaution when running this assay. In addition to the gloves, it is strongly recommended to:

1. Work in a dedicated area or a very clean surface
2. Use dedicated boxes of pipet tips (pipet tips with filter are preferable)
3. Use dedicated materials and consumables for this product
4. All chemicals used to perform this assay should ONLY be used for this kit

5. Sequencing Grade Water should be used for all dilutions and buffer making. MilliQ water is NOT suitable due to potential contamination of DNA and nuclease.
6. Avoid vortexing and excessive pipetting.

Assay Procedure

IMPORTANT: PLEASE READ THE RECOMMENDATIONS BEFORE USE

- The manual described below is an example for generating one standard curve in triplicate and 452 sample wells in a 50 µL final assay volume. 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 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

		Volume				
Format	**# of data points**	**Final**	**Sample**	**AlphaLISA beads / Biotin Antibody MIX**	**SA-Donor beads**	**Plate recommendation**
AL331HV	100	100 µL	10 µL	40 µL	50 µL	White CulturPlate-96 (cat # 6005680)
AL331C	250	100 µL	10 µL	40 µL	50 µL	White CulturPlate-96 (cat # 6005680)
	500	50 µL	5 µL	20 µL	25 µL	White CulturPlate-96 (cat # 6005680) White CulturPlate-384 (cat # 6007680)
	1 250	20 µL	2 µL	8 µL	10 µL	White CulturPlate-384 (cat 6007680)
	2 500	10 µL	1 µL	4 µL	5 µL	White CulturPlate-1536 (cat # 6004680)
AL331F	5 000	50 µL	5 µL	20 µL	25 µL	White CulturPlate-384 (cat # 6007680)
	12 500	20 µL	2 µL	8 µL	10 µL	White CulturPlate-384 (cat # 6007680)
	25 000	10 µL	1 µL	4 µL	5 µL	White CulturPlate-1536 (cat # 6004680)

The manual 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.

Assays are run in CulturPlates-384.

1) Preparation of 1X AlphaLISA DNA Detection Buffer + 0.1% BSA:
Add 10 mL of 10X AlphaLISA DNA Detection Buffer and 2 mL of UltraPure BSA (not supplied with the kit) to 88 mL Sequencing Grade H_2O

2) Preparation of DNA analyte standard dilutions:

Analyte is provided in solution at 10 µg/ml. Prepare standard dilutions as follows in 1X AlphaLISA DNA Detection Buffer (mix solution gently with pipette, excessive pipetting and/or vortexing could break down DNA, change tip between each standard dilution):

Tube	Vol. of DNA (µL)	Vol. of diluent (µL) *	[DNA] in standard curve	
			(g/mL in 5 µL)	(ng/mL in 5 µL)
A	10 µL of provided DNA	90	1.00E-06	1000
B	60 µL of tube A	120	3.33E-07	333.333
C	60 µL of tube B	120	1.11E-07	111.111
D	60 µL of tube C	120	3.70E-08	37.037
E	60 µL of tube D	120	1.23E-08	12.346
F	60 µL of tube E	120	4.12E-09	4.115
G	60 µL of tube F	120	1.37E-09	1.372
H	60 µL of tube G	120	4.57E-10	0.457
I	60 µL of tube H	120	1.52E-10	0.152
J	60 µL of tube I	120	5.08E-11	0.051
K	60 µL of tube J	120	1.69E-11	0.017
L	60 µL of tube K	120	5.65E-12	0.006
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 DNA Detection 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.

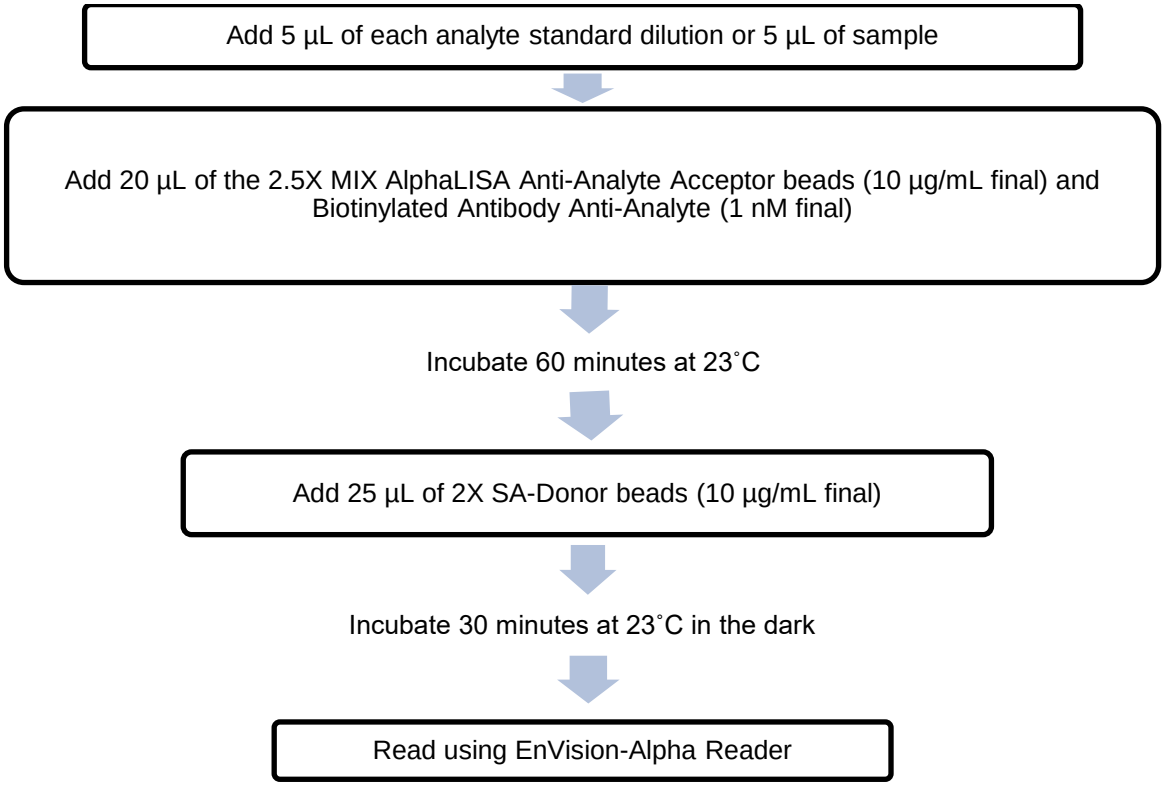
** Four background points in triplicate (12 wells) are used when LDL is calculated. If LDL does not need to be calculated, one background point in triplicate can be used (3 wells).

*** **High background or inconsistent results usually indicate unwanted contamination.**

3) Preparation of 2.5X MIX AlphaLISA Anti-DNA Acceptor beads (25 µg/mL) and Biotinylated Anti DNA Antibody (2.5 nM): Add 50 µL of 5 mg/mL AlphaLISA Anti DNA Acceptor beads and 50 µL of 500 nM Biotinylated Anti DNA to 9.9 mL of 1X AlphaLISA DNA Detection Buffer. Prepare just before use.

4) Preparation of 2X Streptavidin (SA) Donor beads (20 µg/mL): Keep the beads under subdued laboratory lighting.
Add 50 µL of 5 mg/mL SA-Donor beads to 12.45 mL of 1X AlphaLISA DNA Detection Buffer. Prepare just before use.

5) In a white CulturPlate (384 wells):



IMPORTANT:

The provided analyte is highly purified DNA fragment (4000 bp). Performance of this assay on a variety of DNA from different species and of varying lengths has been validated (see below). However, it is recommended to create a standard with the relevant host cell DNA (i.e. use CHO cell DNA standard when detecting CHO cell contamination). This AlphaLISA kit is not optimized for the quantification of DNA fragments <300 bp.

Data Analysis

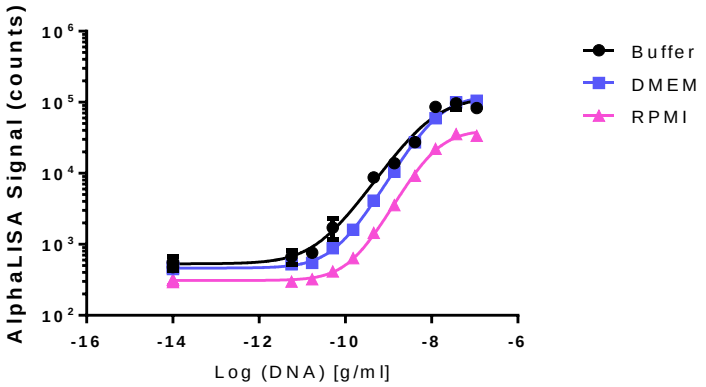
- Calculate the average count value for the background wells.
- Generate a standard curve by plotting the AlphaLISA counts versus the concentration of analyte. A log scale can be used for either or both axes. No additional data transformation is required.
- Analyze data according to a nonlinear regression 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 2 step manual.

- 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 (ng/mL)	Buffer/Media
0.01	AlphaLISA DNA Detection Buffer
0.02	DMEM
0.07	RPMI

* 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. In each lot, the analytes were prepared in AlphaLISA DNA Detection Buffer, 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 AlphaLISA DNA Detection Buffer.

- Intra-assay precision:

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

DNA	DNA Detection Buffer
CV%	12%

- Inter-assay precision:

The inter-assay precision was determined using a total of 3 independent determinations with 9 measurements for 4 ng/mL sample. Shown are CV%.

DNA (4 ng/ml)	DNA Detection Buffer
CV%	17%

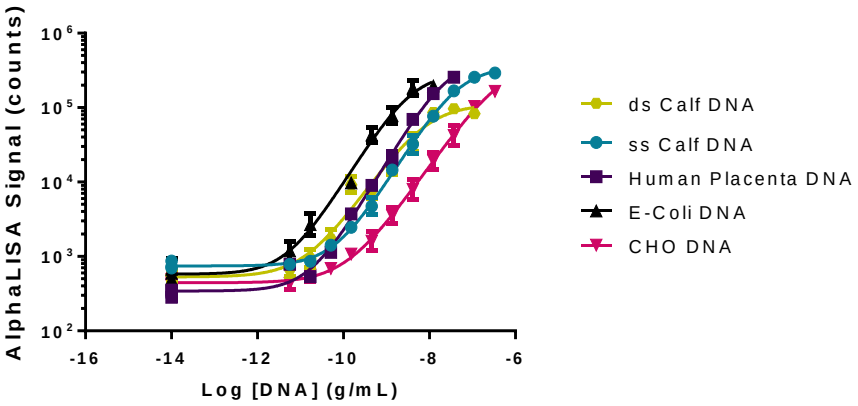
- Spike Recovery:

Three known concentrations of analyte were spiked in DNA Detection Buffer. All samples, including non-spiked DNA Detection Buffers were measured in the assay. The average recovery from three independent measurements is reported.

Spiked DNA (ng/mL)	% Recovery
	DNA Detection Buffer
10	91
1	84
0.1	84

- Species Specificity:

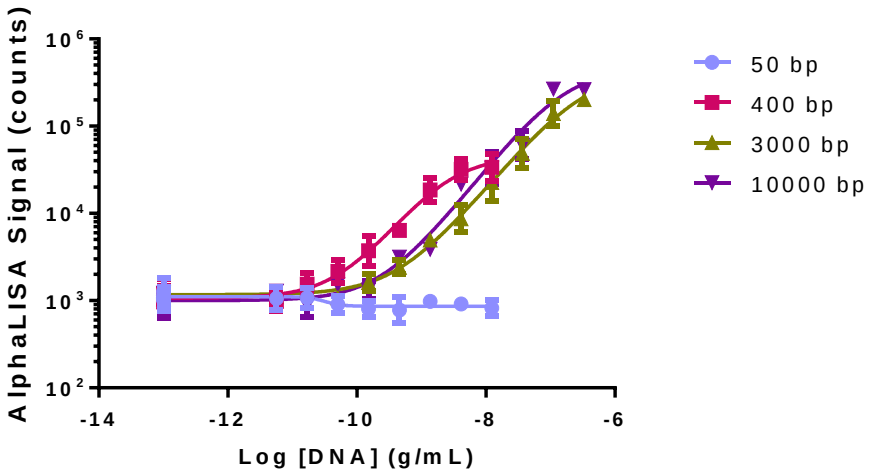
Full sensitivity curves were generated with DNA of different origin, either double- or single-stranded. LDL for individual curves is reported.



Protein	LDL (ng/ml)
ds Calf DNA	0.01
ss Calf DNA	0.02
Human Placenta DNA	0.01
E-Coli DNA	0.01
CHO DNA	0.08

- Performance based on fragment size:

Full sensitivity curves were generated with DNA fragments of different sizes.



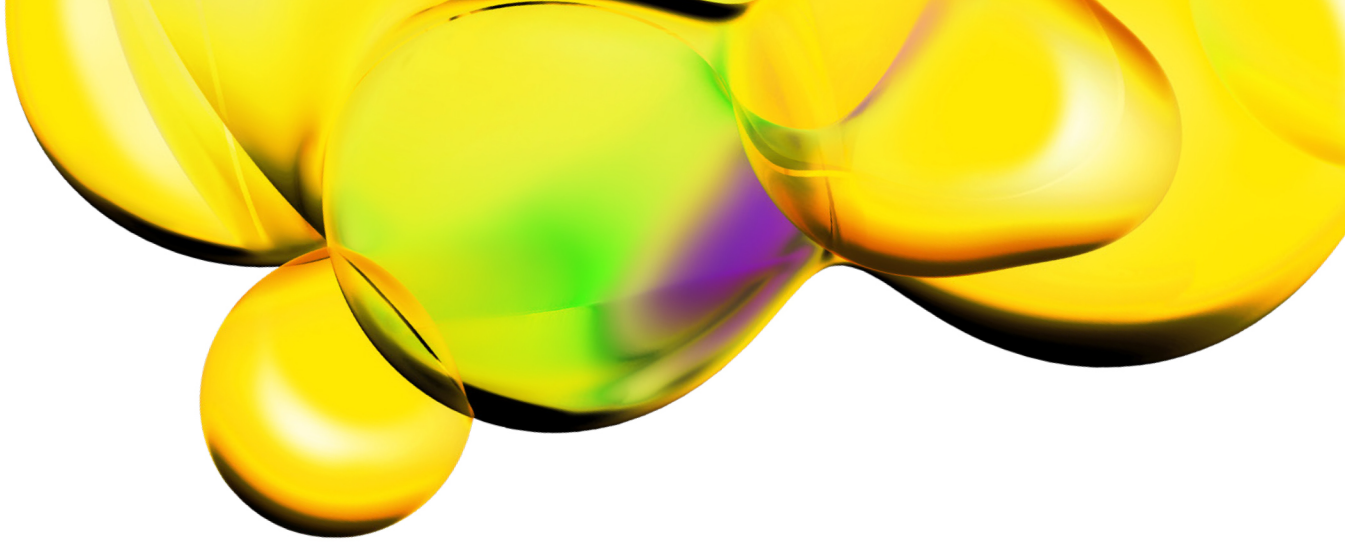
IMPORTANT:

For optimal results, please use DNA Contamination Detection AlphaLISA kit with DNA fragments longer than 400 bp.

Troubleshooting Guide

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

RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.



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