TECHNICAL DATA SHEET

AlphaLISA® Research Reagents

AlphaLISA Somatostatin Detection Kit

Product number: AL329 HV/C/F

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

Contents

Product Information

Application: This kit is designed for the quantitative determination of Somatostatin, using a homogeneous AlphaLISA assay (no wash steps). The assay shows negligible cross-reactivity with other biomolecules of similar structure.

Sensitivity: Lower Detection Limit (LDL): 55 pg/mL

IC_{50}: 500 pg/mL

Dynamic range: 55 – 1 637 000 pg/mL (Figure 1).

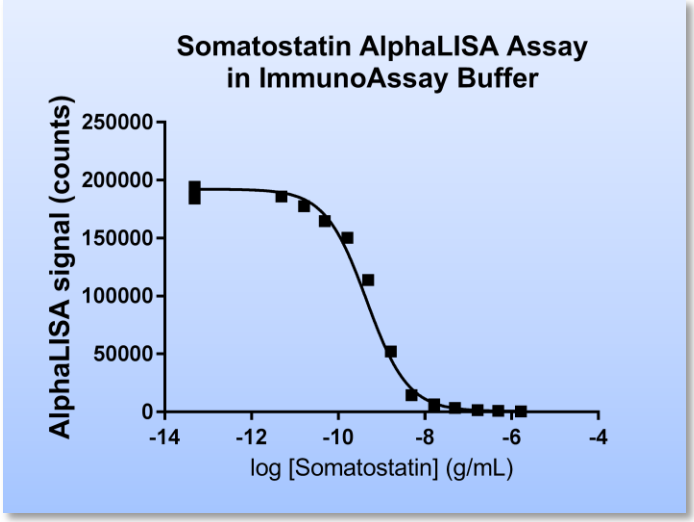


Figure. 1. Typical sensitivity curves in AlphaLISA buffer The data was generated using a white Optiplate™-384 microplate and the EnVision® Multilabel Plate Reader 2103 with Alpha option.

Storage: Store kit in the dark at +4˚C. Reconstituted analyte and tracer can be stored at 4 °C for up to 2 months

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, IC_{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.

Analyte of Interest

Somatostatin is a short peptide hormone (14 or 28 amino acids) involved in regulating the endocrine system. This kit detects both active forms of peptide (Somatostatin-14 and Somatostatin-28). It plays a role in neurotransmission and cell proliferation. Somatostatin interacts with the G protein-coupled Somatostatin receptor to inhibit insulin and glucagon secretion. This peptide exists in almost all vertebrates; coded by six different genes (SS1-6). Classified as an inhibitory hormone, Somatostatin inhibits the release of growth hormone and thyroid-stimulating hormone in the pituitary and multiple gastrointestinal hormones: gastrin, CCK, secretin, and GIP.

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 competition assay, a Biotinylated analog of the analyte of interest, the tracer, binds to the Streptavidin-coated Alpha Donor beads, while the Anti-Analyte Antibody is conjugated to AlphaLISA Acceptor beads. In the presence of low 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). In the presence of high analyte, the beads are separated resulting in lower emission.

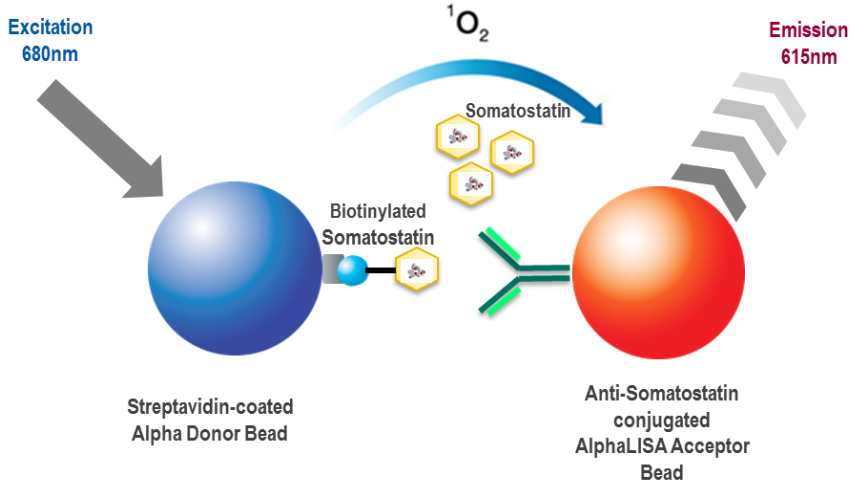


Figure 2. AlphaLISA Assay principle.

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 saliva. Take precautionary measures to avoid contamination of the reagent solutions.

Kit Content: Reagents and Materials

Kit components	AL329HV (100 assay points**)	AL329C (500 assay points**)	AL329F (5 000 assay points**)
AlphaLISA Acceptor beads stored in PBS, 0.05% Proclin-300, pH 7.2	50 µL @ 5 mg/mL (1 brown tube, white cap)	110 µL @ 5 mg/mL (1 brown tube, white cap)	1100 µL @ 5 mg/mL (1 brown tube, white cap)
Streptavidin (SA)-coated Donor beads stored in 25 mM HEPES, 100 mM NaCl, 0.05% Proclin-300, pH 7.4	50 µL @ 5 mg/mL (1 brown tube, black cap)	110 µL @ 5 mg/mL (1 brown tube, black cap)	1100 µL @ 5 mg/mL (1 brown tube, black caps)
Biotinylated Somatostatin-Tracer Solid	2 nmol (1 tube, black cap)	2 nmol (1 tube, black cap)	2 nmol (1 tube, black cap)
Somatostatin Analyte Standard Solid	2 nmol (1 tube, clear cap)	2 nmol (1 tube, clear cap)	2 nmol (1 tube, clear cap)
AlphaLISA ImmunoAssay Buffer (10X) *	2 mL, 1 small bottle	10 mL, 1 small bottle	100 mL, 1 large bottle

* 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 or 50 µL 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 Adhesive Sealing Film	PerkinElmer Inc.	6050195
EnVision®-Alpha Reader	PerkinElmer Inc.	-
1 X PBS, pH 7.4	Gibco	10010

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 solid analyte) before use to improve recovery of content (2000g, 10-15 sec).

Re-suspend all reagents by vortexing before use.

- Use 1X PBS pH 7.4 without Calcium or Magnesium when reconstituting the Biotinylated Somatostatin-Tracer and the Somatostatin Analyte Standard (ex: Gibco cat# 10010)
- 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. It is recommended to the spin the microplate (1000 rpm; 1 min) prior to incubations.
- 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 a similar matrix as the samples (e.g. charcoal stripped serum for serum samples).
- AlphaLISA assays can be performed in cell culture medium with or without phenol red, with the following recommendations: if possible, avoid biotin-containing medium (e.g. RPMI medium) as lower counts and lower sensitivity are expected. Add at least 1% FBS or 0.1% BSA to cell culture medium.

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 MAX points in triplicate (12 wells) is recommended when LDL is calculated. One MAX point in triplicate (3 wells) can be used when LDL is not calculated
- Use of null (MIN) points in triplicate (12 wells) is recommended when normalizing data. Analyzing true signal data does not require null points

		Volume					
Format	**# of data points**	**Final**	**Sample**	**AlphaLISA Acceptor Bead**	**Biotinylated Tracer**	**SA-Donor beads**	**Plate recommendation**
AL329HV	100	100 µL	40 µL	20 µL	20	20 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
AL329C	250	100 µL	40 µL	20 µL	20 µL	20 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
	500	50 µL	20 µL	10 µL	10 µL	10 µL	White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate™-384 (cat # 6005350)
	1 250	20 µL	8 µL	4 µL	4 µL	4 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate™-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	2 500	10 µL	4 µL	2 µL	2 µL	2 µL	Light gray AlphaPlate-1536 (cat # 6004350)
AL329F	5 000	50 µL	20 µL	10 µL	10 µL	10 µL	White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate-384 (cat # 6005350)
	12 500	20 µL	8 µL	4 µL	4 µL	4 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	25 000	10 µL	4 µL	2 µL	2 µL	2 µL	Light gray AlphaPlate-1536 (cat # 6004350)

Standard protocol (3 incubation steps) – Dilution of standards in 1X AlphaLISA ImmunoAssay Buffer, cell culture media or Charcoal Stripped Serum

The protocol described below is recommended for generating one standard curve in a 50 µL final assay volume (48 wells, triplicate determinations with manual pipetting) and 452 sample 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:
    a. Add 2 mL of 10X AlphaLISA ImmunoAssay Buffer to 18 mL H_2O.
2) Preparation of Somatostatin analyte standard dilutions:
    a. Reconstitute Somatostatin analyte standard (2 nmol) in 200 µL of Milli-Q Water to 10 µM (16,370 ng/mL). The first point of the curve is 1 µM (1637 ng/mL), so a 10-fold dilution of the solution is required.
    b. Prepare standard dilutions as follows (change tip between each standard dilution) in 1X AlphaLISA ImmunoAssay Buffer, cell culture medium, or charcoal stripped serum:

Tube	Vol. of Somatostatin (µL)	Vol. of diluent (µL) *	[Somatostatin] in standard curve	
			(µM in 20 µL)	(ng/mL in 20 µL)
A	20 µL of Somatostatin std	180	1	1637
B	60 µL of tube A	140	0.3	491
C	60 µL of tube B	120	0.1	164
D	60 µL of tube C	140	0.03	49
E	60 µL of tube D	120	0.01	16
F	60 µL of tube E	140	0.003	5
G	60 µL of tube F	120	0.001	1.6
H	60 µL of tube G	140	0.0003	0.5
I	60 µL of tube H	120	0.0001	0.16
J	60 µL of tube I	140	0.00003	0.05
K	60 µL of tube J	120	0.00001	0.016
L	60 µL of tube K	140	0.000003	0.005
M ** (MAX)	0	100	0	0
N ** (MAX)	0	100	0	0
O ** (MAX)	0	100	0	0
P ** (MAX)	0	100	0	0
Q *** (null)	0	100	0	0
R *** (null)	0	100	0	0
S *** (null)	0	100	0	0
T*** (null)	0	100	0	0

* Dilute standards in diluent (e.g. 1X AlphaLISA ImmunoAssay Buffer, cell culture medium, charcoal stripped serum). 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. These represent the MAX signal in the assay. If LDL does not need to be calculated, one background point in triplicate can be used (3 wells).

*** Four null points in triplicate (12 wells) are used to determine the MIN signal of the assay (lowest possible counts). These wells contain anti-Somatostatin Acceptor Bead, SA-Donor Bead, and Buffer.

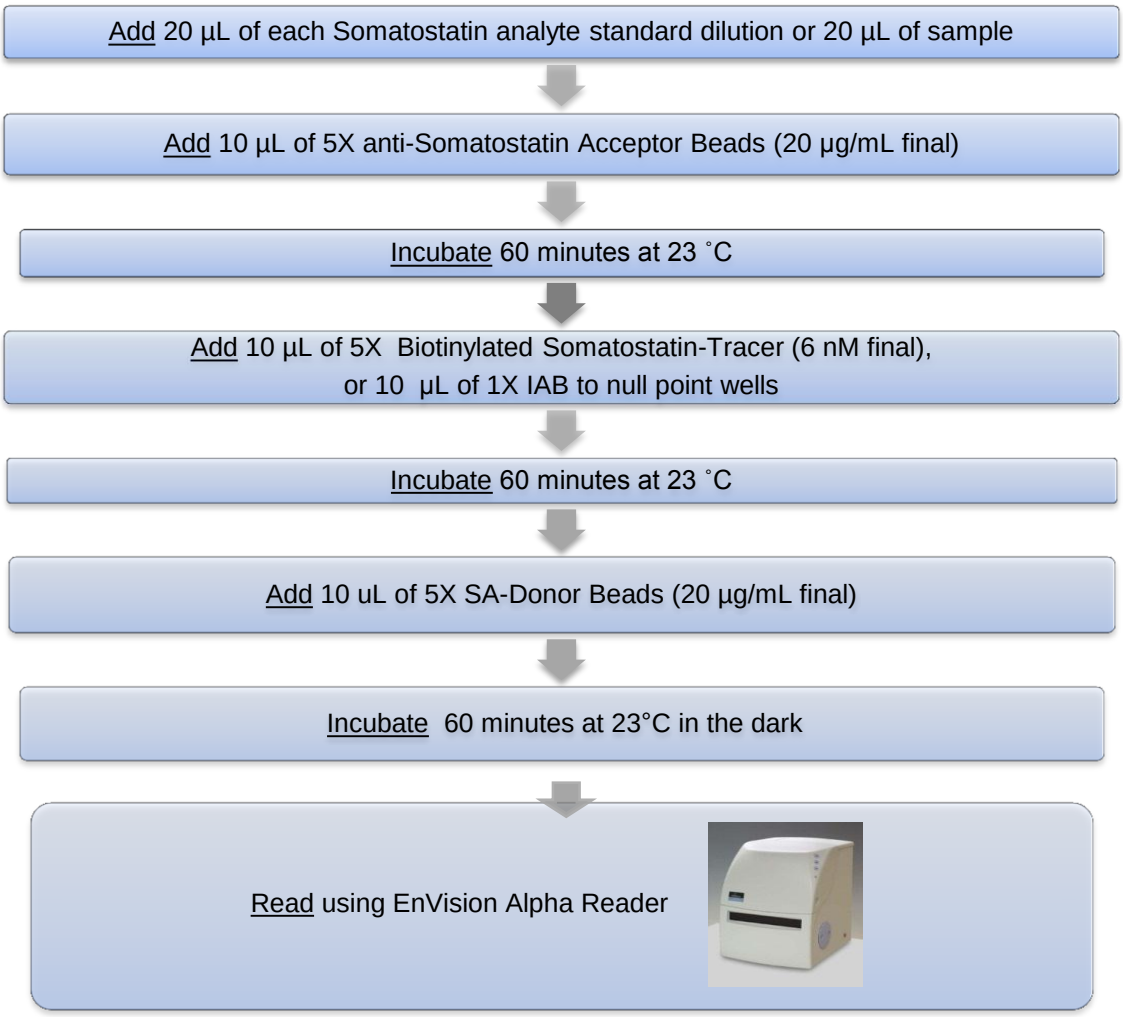
3) Preparation of 5X AlphaLISA Anti-Somatostatin Acceptor beads (100 µg/mL):
   a. Add 100 µL of 5 mg/mL AlphaLISA Anti-Somatostatin Acceptor beads to 4900 µL of 1X AlphaLISA ImmunoAssay Buffer.
   b. Prepare just before use

4) Preparation of 5X Biotinylated Somatostatin-Tracer (30 nM):
   a. Dissolve 2 nmol vial of Biotinylated Somatostatin-Tracer in 1.0 mL Milli-Q water. Add 75 µL of 2 µM Biotinylated Somatostatin-Tracer solution to 4925 µL of 1X AlphaLISA Immunoassay Buffer.
   b. Prepare just before use
   c. For Null points, add 10 µL per well of Immunoassay Buffer ONLY to Null point wells.

5) Preparation of 5X Streptavidin (SA) Donor beads (100 µg/mL):
   a. Keep the beads under subdued laboratory lighting!
   b. Add 100 µL of 5 mg/mL SA-Donor beads to 4900 µL of 1X AlphaLISA ImmunoAssay Buffer.
   c. Prepare just before use.

6) Samples:
   a. If applicable, dilute samples to be tested in diluent (e.g. 1X AlphaLISA ImmunoAssay Buffer).

7) In a 96- or 384-well micro plate (**It is recommended to spin the plate before incubations, 1000 rpm; 1 min)**:



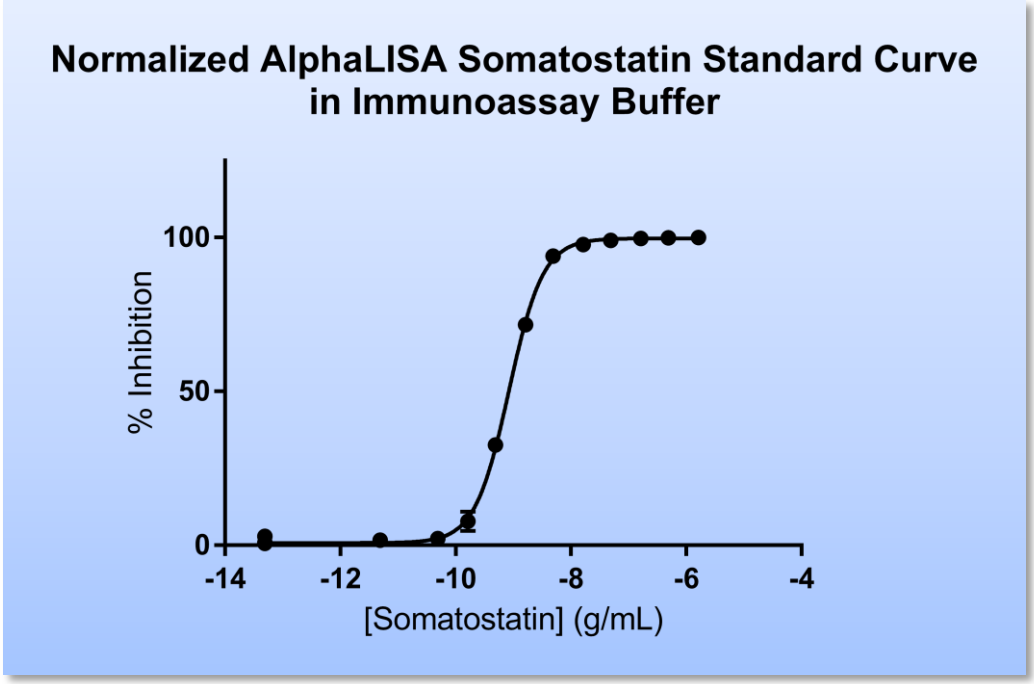
Data Analysis (Direct)

- 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 MAX counts (12 wells without analyte) - 3 x standard deviation value (average MAX counts - (3xSD)) on the standard curve.
- 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.

Data Analysis (Normalized)

- The Somatostatin AlphaLISA Competition Assay is strongly dependent on tracer concentration. Small changes in concentration can strongly affect top and bottom counts.

- It is recommended to convert data to normalized response. This requires a set of “null points” These wells contain only AlphaLISA Anti-Somatostatin Acceptor Bead AlphaLISA SA-Donor Bead and buffer made up to a volume consistent with the standard curve.
- Calculate the average count value for the “null points” (12 wells without analyte or tracer).
- Generate a standard curve by plotting the AlphaLISA counts versus the concentration of analyte. A log scale can be used for the X-axis
- Normalize the data using the following equation (1-((sample counts – Avg (null points))/ Avg (MAX)))*100
- Analyze data according to a nonlinear regression using the 4-parameter logistic equation (sigmoidal normalized dose-response curve with variable slope) ***Do not weight this data***
- Data will be presented as % inhibition (percentage of tracer inhibited by analyte).
- The LDL is calculated by interpolating the average normalized MAX counts (12 wells without analyte) + 3 x standard deviation value (average normalized MAX counts + (3xSD)) on the standard curve.
- 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 standard protocol.

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 20 µL using the recommended assay conditions.

LDL (pg/mL)	Buffer/Media used	# of experiments
55	AlphaLISA ImmunoAssay Buffer	9
81	DMEM Culture Media	6
200	Charcoal stripped Serum	6

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 ImmunoAssay Buffer (IAB) and charcoal stripped serum. 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 ImmunoAssay Buffer.

- Intra-assay precision:

The intra-assay precision was determined using a total of 10 independent determinations in triplicate. Shown CV%.

Somatostatin (pg/ml)	ImmunoAssay Buffer	DMEM	Charcoal Stripped Serum
500	8	9	11
5000	4	6	7

- Inter-assay precision:

The inter-assay precision was determined using a total of 6 independent determinations. Shown CV%.

Somatostatin (pg/ml)	ImmunoAssay Buffer
5000	8

b. Spike and Recovery:

Independent samples were tested against standard curves prepared in the respective media. Shown as %Recovery

Somatostatin (ng/mL)	Immunoassay Buffer	DMEM Culture Media	Charcoal Stripped Serum
164	83	88	95
25	113	103	116
4	115	98	89

- Specificity:

Cross-reactivity of the AlphaLISA Somatostatin Kit was tested using the following biomolecules at 5 ng/mL in AlphaLISA ImmunoAssay Buffer:

Biomolecule	% Cross-reactivity
Somatostatin-28	100
Octreotide	<1%

Troubleshooting Guide

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

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

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