MANUAL

Technology: AlphaLISA®

AlphaLISA Human and Mouse Ataxin-2 Detection Kit

Part number:	AL3193HV	AL3193C	AL3193F
Assay points:	100	500	5,000

Storage: Store kit in the dark at 4 °C. For reconstituted analyte, aliquot and store at -20 °C. Avoid freeze-thaw cycles.

Version: 1 Date: August 2023

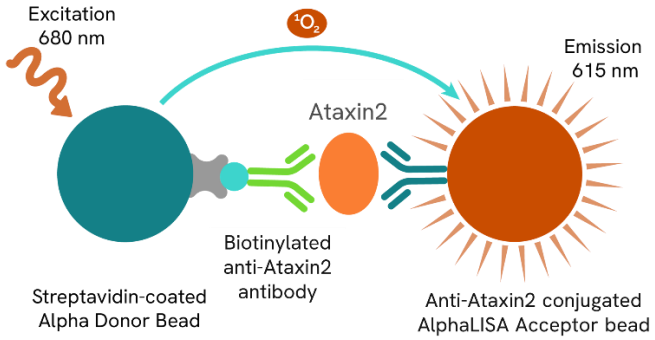
ANALYTE OF INTEREST

Ataxin-2 is an RNA-binding protein. This protein is ubiquitous protein that can be found in different species, in different organs (but mostly the brain) or at different location in the cells. In humans, ataxin-2 causes neurodegeneration when carrying very long polyglutamine tract and drives disease progression in Amyotrophic lateral sclerosis (ALS) or Spinocerebellar ataxia type 2 (SCA2). Moreover, ataxin-2 absence or loss-of-function promotes obesity and weight gain.

Current research in neurodegenerative and specifically ALS settings indicates there is a modulation of ataxin-2 expression by TDP-43, which in turns sees its toxicity in cellular and animal models modified by ataxin-2. The co-investigation of both proteins is a relevant topic for future therapeutic endeavors, with recent results indicating that lowered ataxin-2 levels suppress TDP-43 aggregation.

DESCRIPTION OF THE ALPHALISA ASSAY

AlphaLISA technology allows the detection of molecules of interest in buffer, cell culture media and serum in a highly sensitive, quantitative, reproducible and user-friendly mode. In this AlphaLISA assay, a biotinylated anti-Ataxin-2 antibody binds to the streptavidin coated AlphaLISA Donor beads, while the anti-Ataxin-2 antibody is conjugated to AlphaLISA Acceptor beads. In the presence of ataxin- 2, the beads come into proximity. The excitation of the Donor beads provokes the release of singlet oxygen molecules that triggers a cascade of energy transfer within the Acceptor beads, resulting in emission with λmax at 615 nm.



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) can be applied to light fixtures.
- Take precautionary measures to avoid contamination of the reagent solutions.
- The biotinylated anti-Ataxin 2 antibody contains sodium azide. Contact with skin or inhalation should be avoided.

KIT CONTENT: REAGENTS AND MATERIALS

Kit components	AL3193HV***	AL3193C****	AL3193F****
AlphaLISA Anti-Ataxin-2 Acceptor beads stored in PBS, 0.05% Kathon CG/ICP, pH 7.2	25 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)
Streptavidin (SA)-coated Donor beads stored in 25 mM HEPES, 100 mM NaCl, 0.05% Kathon CG/ICP II, pH 7.4	100 µL @ 5 mg/mL (1 brown tube, black cap)	200 µL @ 5 mg/mL (1 brown tube, black cap)	2 x 1mL @ 5 mg/mL (x brown tubes, black caps)
Biotinylated Anti-Ataxin-2 Antibody stored in PBS, 0.1% Tween-20, 0.05% NaN3, pH 7.4	75 µL @ 500 nM (1 tube, black cap)	150 µL @ 500 nM (1 tube, black cap)	2x750 µL @ 500 nM (1 tube, black cap)
AlphaLISA Ataxin-2 Lyophilized analyte *	4 ng (5 tube, clear cap)	4 ng (5 tube, clear cap)	4 ng (5 tube, clear cap)
AlphaLISA Hiblock Buffer** (10X)	2 mL, 1 small bottle	10 mL, 1 medium bottle	100 mL, 1 large bottle
AlphaLISA Ataxin-2 Lysis Buffer (4X)	2 mL, 1 small bottle	10 mL, 1 medium bottle	100 mL, 1 large bottle

* Reconstitute lyophilized analyte in 200 µL Milli-Q® grade H_2O. IMPORTANT: do not vortex the analyte. The reconstituted 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 4 weeks. Avoid freeze-thaw cycles. One vial contains an amount of analyte sufficient for performing 2 standard curves. Additional vials can be ordered separately (cat # AL3193S).

** Extra buffer can be ordered separately (cat # AL004C: 10 mL, cat # AL004F: 100 mL).

*** The number of assay points is based on an assay volume of 100 µL in 96-well plates.

**** The number of assay points is based on an assay volume of 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. Note that sodium azide from the biotinylated anti-Ataxin-2 antibody stock solution will not interfere with the AlphaLISA signal (0.0001% final in the assay).

Additional reagents and materials:

The following materials are recommended but not provided in the kit:

Item	Suggested source
Light gray AlphaPlate™-384	Revvity Inc.
TopSeal™-A Plus Adhesive Sealing Film	Revvity Inc.
EnVision®-Alpha Reader	Revvity Inc.

RECOMMENDATONS

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 (2000*g*, 10-15 sec).
- Re-suspend the Donor and Acceptor beads by vortexing before use.
- Use Milli-Q® grade H_2O to dilute buffers and to reconstitute the lyophilized analyte. Do not vortex the 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 Film to reduce evaporation during incubation. Microplates can be read with the TopSeal-A Film in place.
- The AlphaLISA signal is detected with an EnVision Multimode 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. It is recommended to avoid multiple reads of the same well of the assay plate.
- The standard curves shown in this manual are provided for information only. A standard curve must be generated for each experiment.

ASSAY PROCEDURE

- 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 different amounts of samples are tested, the volumes of all reagents must 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.

Format	# of data points	Volumes for 2-Step High Concentration Protocol				Plate recommendation
		Final	Sample	AlphaLISA Acceptor Beads + biotinylated Ab Mix	SA-Donor beads	
AL3193HV	100	100 µL	10 µL	10 µL	80 µL	White OptiPlate-96 White ½ AreaPlate-96
AL3193C	250	100 µL	10 µL	10 µL	80 µL	White OptiPlate-96
	500	50 µL	5 µL	5 µL	40 µL	½ Area AlphaPlate-96 White OptiPlate-384 Light gray AlphaPlate™-384
	1 250	20 µL	2 µL	2 µL	16 µL	Light gray AlphaPlate-384 ProxiPlate™-384 Plus White OptiPlate-384
	2 500	10 µL	1 µL	1 µL	8 µL	Light gray AlphaPlate-1536
AL3193F	5 000	50 µL	5 µL	5 µL	40 µL	½ Area AlphaPlate-96 White OptiPlate-384 Light gray AlphaPlate-384
	12 500	20 µL	2 µL	2 µL	16 µL	Light gray AlphaPlate-384 ProxiPlate-384 Plus White OptiPlate-384
	25 000	10 µL	1 µL	1 µL	8 µL	Light gray AlphaPlate-1536

The 2-Step High Concentration protocol described below is for 500 assay points including one standard curve (48 wells) and samples (452 wells). *If different amounts of samples are tested, the volumes of all reagents must be adjusted accordingly.*

1) Preparation of 1X AlphaLISA Ataxin-2 Lysis Buffer:
Add 1.25 mL of 4X AlphaLISA Ataxin-2 Lysis Buffer to 3.75 mL Milli-Q® grade H_2O.

2) Preparation of Ataxin-2 analyte standard dilutions:

   a. Reconstitute lyophilized Ataxin-2 (4 ng) in 200 µL Milli-Q® grade H_2O. The remaining reconstituted analyte should be aliquoted immediately and stored at -20 °C for future assays (see page 2 for more details).
   b. A standard curve must be generated for each experiment. The standard curve should be performed in a similar matrix diluent as the samples (e.g. cell culture media for cell supernatant samples, FBS for serum samples). Use of the 1X AlphaLISA Ataxin-2 Lysis buffer is recommended as a diluent to confirm assay performance.
   c. Prepare standard dilutions as follows (change tip between each standard dilution).

Tube	Vol. of Ataxin 2 (µL)	Vol. of diluent (µL)*	[Ataxin-2] in standard curve	
			(g/mL in 5 µL)	(pg/mL in 5 µL)
A	80 µL of reconstituted Ataxin-2 standard)	_	2.00E-08	20,000
B	60 µL of tube A	140	6.00E-09	6,000
C	60 µL of tube B	120	2.00E-09	2,000
D	60 µL of tube C	140	6.00E-10	600
E	60 µL of tube D	120	2.00E-10	200
F	60 µL of tube E	140	6.00E-11	60
G	60 µL of tube F	120	2.00E-11	20
H	60 µL of tube G	140	6.00E-12	6
I	60 µL of tube H	120	2.00E-12	2
J	60 µL of tube I	140	6.00E-13	0.6
K	60 µL of tube J	120	2.00E-13	0.2
L	60 µL of tube K	140	6.00E-14	0.1
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 appropriate diluent (e.g. 1X AlphaLISA Ataxin-2 Lysis 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 1X AlphaLISA HiBlock Buffer:
Add 5 mL of 10 X AlphaLISA HiBlock Buffer to 45 mL Milli-Q® grade H_2O.

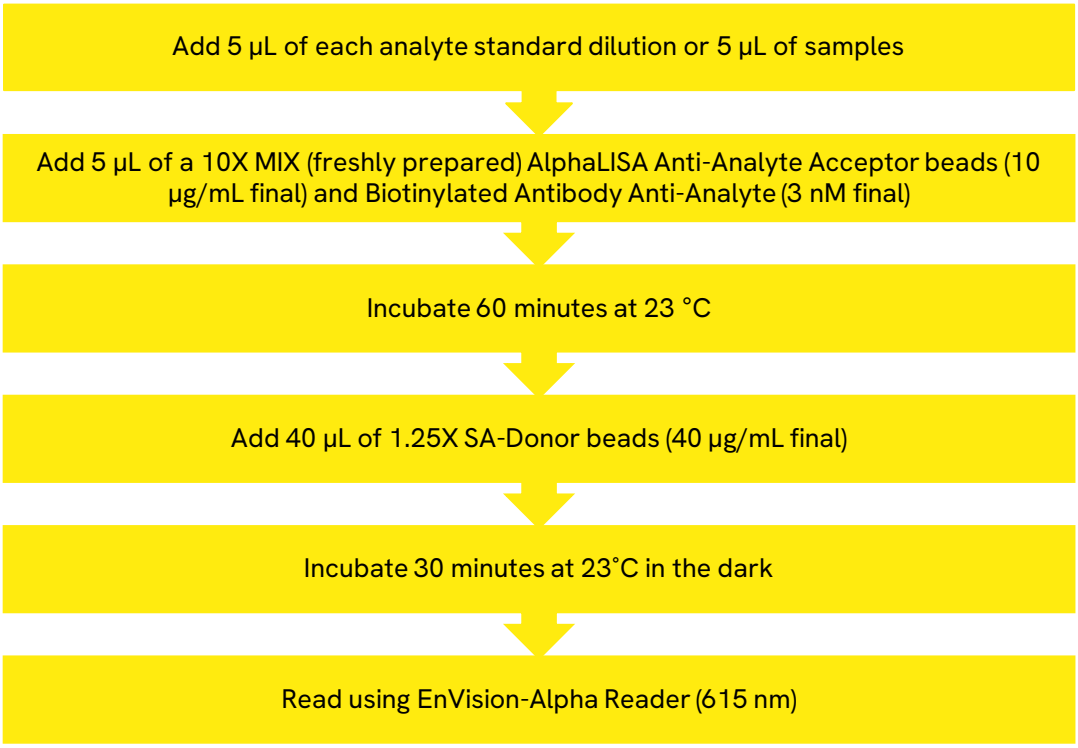
4) Preparation of 10X MIX AlphaLISA Anti-Ataxin-2 Acceptor beads (100 µg/mL) + Biotinylated Anti-Ataxin2 Antibody (30 nM):
   a. Prepare just before use.
   b. Add 50 µL of 5 mg/mL AlphaLISA Anti-Ataxin-2 Acceptor beads and 150 µL of 500 nM Biotinylated Antibody Anti- Ataxin-2 to 2 300 µL of 1X AlphaLISA HiBlock Buffer.

5) Preparation of 1.25X Streptavidin (SA) Donor beads (50 µg/mL):

a. Prepare just before use.
b. Keep the beads under subdued laboratory lighting.
c. Add 200 µL of 5 mg/mL SA-Donor beads to 19 800 µL of 1X AlphaLISA HiBlock Buffer.

6) Samples: If applicable, dilute samples to be tested in Ataxin-2 Lysis buffer (e.g. 1X AlphaLISA Ataxin-2 Lysis Buffer.

5) In a light gray AlphaPlate (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).

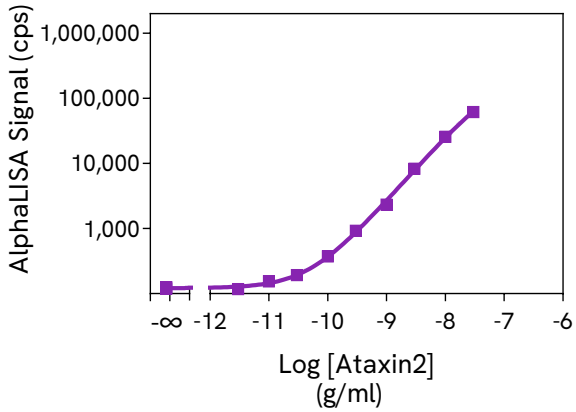
- Read from the standard curve the concentration of analyte contained in the samples.
- If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

ASSAY PERFORMANCE CHARACTERISTICS

AlphaLISA assay performance described below was determined by using a 2-step high concentration protocol using 1X AlphaLISA Ataxin-2 Lysis Buffer as an assay buffer. The analytes (standards) were prepared in Ataxin-2 Lysis buffer. All other components were prepared in 1X AlphaLISA HiBlock Buffer.

Standard curve:

A typical sensitivity curve is shown below, using the 2-step high concentration protocol described on page 4, using 1X AlphaLISA Ataxin-2 lysis Buffer to dilute the standard.



Assay sensitivity:

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

Analyte diluent	*LDL (pg/mL)*	LLOQ (pg/mL)
Ataxin 2 Lysis Buffer	9.0	26.5
100% FBS	11.8	43.6

Assay precision:

The following assay precision data were calculated from three independent assays. The analytes were prepared in Ataxin-2 Lysis buffer. All other components were prepared in AlphaLISA HiBlock Buffer.

Each assay consisted of one standard curve comprising 12 data points (each in triplicate) and 12 background wells (no analyte). The assays were performed in a 384-well plate format.

Intra-assay precision:

The intra-assay precision was determined using a total of 24 independent determinations in triplicate. Shown as CV% of measured concentration. 24 independent determinations of 3 samples at 600, 2000 and 6000 pg/mL. Results are shown as CV% as measured [Ataxin2] concentrations.

Human Ataxin 2 targeted CONCENTRATION	Ataxin-2 estimated concentration (pg/mL)	CV %
Sample A, 600 pg/mL	514	7%
Sample B, 2000 pg/mL	1883	6%
Sample C, 6000 pg/mL	6615	6%

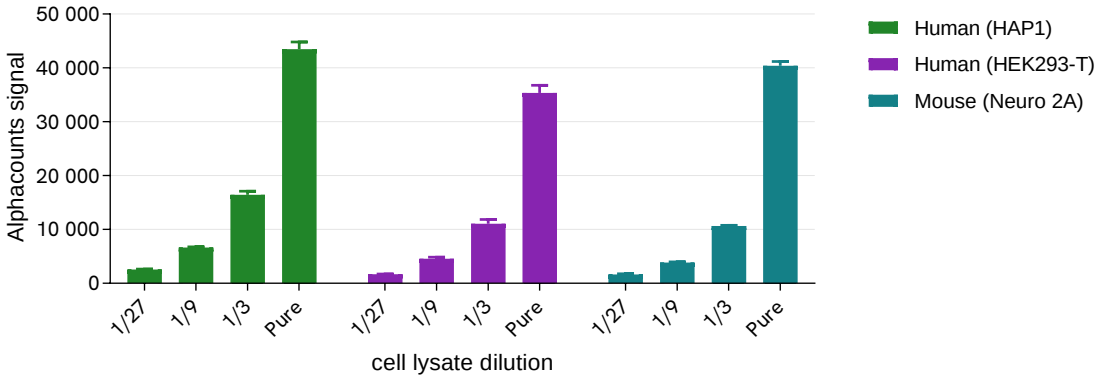
Inter-assay precision:

The inter-assay precision was determined using a total of 3 independent determinations with 6 measurements for 3 samples at 600, 2000 and 6000 pg/mL. Results are shown as CV% as measured [Ataxin2] concentrations.

Human Ataxin-2 targeted CONCENTRATION	Ataxin-2 estimated concentration (pg/mL)	CV %
Sample A, 600 pg/mL	543	10%
Sample B, 2000 pg/mL	1926	10%
Sample C, 6000 pg/mL	6697	8%

Specificity:

The cross-reactivity of the AlphaLISA Ataxin-2 Detection Kit was tested using the 2 human cell lines HAP1 & HEK293T and the mouse cell line Neuro 2A cells lysed in Ataxin-2 lysis buffer. An equivalent signal is detected in human & mouse cell lines.



Proteins	Cross reactivity (%)
Mouse	100%

TROUBLESHOOTING

To find detailed recommendations for common situations you might encounter with your AlphaLISA assay kit, please visit our website at www.revvity.com.

www.revvity.com

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