AlphaLISA FAQ

1-What is the main difference between AlphaLISA® and AlphaScreen®?

The main difference between the two assays is the acceptor beads. With AlphaScreen acceptor beads, final emission is from rubrene; with AlphaLISA acceptor beads, the final emission is from Europium. Therefore, emission wavelength is different between AlphaScreen beads (520-620 nm) and AlphaLISA beads (615 nm). Because of the narrow emission spectrum of AlphaLISA beads, there is much less interference in serum and plasma samples and better sensitivity is obtained.

Note: There is no need to change filters in the EnVision to read AlphaLISA signal.

2-How large are AlphaLISA beads? Will they clog my liquid handling tips?

AlphaLISA beads are small beads (250-350 nm diameter). They will not clog liquid handling tips.

3-What about light sensitivity of Alpha beads?

Streptavidin-coated Alpha donor beads are light sensitive: after excitation at 680 nm, the donor beads release singlet oxygen which then activates the Acceptor beads. Direct sunlight and intense artificial light can also activate donor beads. In that case, singlet oxygen can react with streptavidin bound to the donor-bead, which decreases its efficiency. It is suggested that you work under subdued laboratory lighting of less than 100 Lux. Alternatively, green filters can be applied to light fixtures. Incubations of AlphaLISA assays should be performed in the dark. Plates can be covered by an opaque microplate to minimize the effect of light.

AlphaLISA Acceptor beads show no light-sensitivity.

4-Which analytes can be measured using the Alphalisa kits?

Presently we have eight kits for research on the following analytes:
Insulin, VEGF, Aβ40, Aβ42, IgG, EPO, TNFα and HIV p24. Please note that AlphaLISA kits are for research purposes only and not for use in diagnostic procedures.

5-The analyte that I am interested in measuring is not on your website – do you have it?

We only have the indicated analytes at the present. But your feedback is very important for us. Please let us know which analyte you would like to suggest for the PerkinElmer AlphaLISA development list.

If your analyte of interest is not listed, we can develop a custom assay to your specfications, via our OnPoint Reagent Services.

PerkinElmer also sells different type of acceptor and donor beads and you can develop your own assay by combining different types of acceptor and donor beads

6-How much volume of sample can be used for an AlphaLISA assay?

Assays have been developed using as little as 5 µl of sample, but users can employ higher volumes to increase sensitivity (for matrices other than serum and plasma).

7-How can I prepare analyte-free serum?

The following protocol can be used for serum immunodepletion

MATERIAL:
Streptavidin Sepharose: GE Healthcare, cat# 17-5113-01
Human serum: Cambrex, cat# 14-402E

DAY 1
Calculations:

- Determine the amount of analyte to be removed from the serum or plasma (from literature or other assays)
- Use 100x fold biotinylated antibody than analyte
- Use 20x more biotin-binding sites than biotin

Streptavidin Sepharose preparation:

- Gently shake the bottle of SA-Sepharose to resuspend the matrix (do not vortex!).
- Sediment the matrix by centrifugation at 2000 rpm for 5 minutes. Carefully decant the supernatant.
- Wash the SA-Sepharose by adding 10 mL of 1x PBS. Invert to mix.
- Sediment the matrix by centrifugation at 2000 rpm for 5 minutes.

- Repeat washing and centrifugation steps twice and resuspend in PBS
- Add antibody and incubate for 2 hours at 4°C (with shaking).

Serum depletion:

- Sediment the matrix by centrifugation at 2000 rpm for 5 minutes. Carefully decant the supernatant.
- Wash the SA-Sepharose by adding 10 ml PBS. (Note: Resuspend the matrix by inverting the tube; do not vortex.)
- Sediment the matrix by centrifugation at 2000 rpm for 5 minutes. Carefully decant the supernatant.
- Repeat washing and centrifugation steps once.
- Add serum or plasma sample and resuspend gently the matrix.
- Incubate 18h-24h with agitation at 4°C.

DAY 2: Serum recovery:

- Distribute the SA-Sepharose / serum mix into Eppendorf tubes.
- Sediment the matrix by centrifugation at 12,000 rpm for 10 minutes.
- Recover as much sample as possible and transfer into new tubes.
- Repeat centrifugation step.
- Recover as much sample as possible without aspirating any of the remaining SA-Sepharose and transfer into new tubes.
- Sediment any remaining matrix by centrifugation at 13,000 rpm for 10 minutes.
- Collect the depleted serum or plasma and store at -20ºC.

8-What is the species selectivity for each analyte?

AlphaLISA One Species Selectivity Table:

Kit	Human	Mouse	Rat	Bovine	Porcine	Dog	Chimpanzee	Orangutan	Goat	Rabbit
Ab 40	w	w	w	**SS**	**SS**	**SS**	**SS**	**SS**		**SS**
Ab 42	w	w	w	**SS**	**SS**	**SS**	**SS**	**SS**		**SS**
Insulin	w	w	w	w	w	**SS**	**SS**	**SS**	**C**	**SS**
TNFa	w	***x***	***x***	***x***			**C**	**C**		
VEGF*	w	***x***	***x***	**C**	**C**					
EPO	w	***x***	***x***				**C**			
IgG	w	***x***	***x***						***x***	***x***

W ***Worked***
x ***Tested, did not work***
SS ***Same sequence as human or other species tested***

C ***Close sequence as human or other species tested***
******* ***VEGF* = human VEGF-A***

9-What is the LLD and dynamic range of each analyte?

Kit	Buffer LLD	Serum LLD	HDL	Dynamic Range
Aß40	88 pg/mL	ND	100,000 pg/mL	3 logs
Aß42	300 pg/mL	ND	300,000 pg/mL	3 logs
EPO	1 mIU/mL	5.8 mIU/mL	30,000 mIU/mL	> 4 logs
HIV p24	0.52 ng/mL	ND	3,000 ng/mL	> 3.5 logs
IgG	0.24 ng/mL	ND	1,000 ng/mL	> 3.5 logs
Insulin	0.8 µIU/mL	1.3 µIU/mL	3,000 µIU/mL	> 3.5 logs
TNFα	2.2 pg/mL	3.6 pg/mL	30,000 pg/mL	> 4 logs
VEGF	2.2 pg/mL	10.7 pg/mL	100,000 pg/mL	> 4.5 logs

10-Which matrix should be used for the assay?

Kit	**Buffer**	**Serum**	**Plasma**	**Cell culture Medium**
Aß40	**w**	**ND**	**ND**	**w**
Aß42	**w**	**ND**	**ND**	**w**
EPO	**w**	**w**	**w**	**w**
HIV p24	**w**	**ND**	**ND**	**w**
IgG	**w**	**ND**	**ND**	**w**
Insulin	**w**	**w**	**w**	**w**
TNFα	**w**	**w**	**w**	**w**
VEGF	**w**	**w**	**w**	**w**

W ***Worked***
ND ***Not determined***

11-How much total volume of serum can be used in the assay?

Due to the fact that the hemoglobin of the serum will capture some of the oxygen released by the donor beads the percentage of serum should not exceed 10% of the total volume (i.e. 5 µl serum sample in 50 µl final assay volume).

Only the following AlphaLISA kits were tested using serum samples :
Insulin
TNFα
VEGF
EPO

13-How do I calculate the LLD?

Test the blank 12 times within the same run. LLD is mean ± 3 standard deviations when read on the standard curve.

14- How can I increase the sensitivity of my assay?

Sensitivity can be increased by following the high sensitivity protocols given our TD sheets (when applicable). Another way to get higher sensitivity is by using higher volume of the sample or by incubating for longer time.

15- How can I decrease sensitivity of my assay?

You can decrease the sensitivity by adding less sample (i.e. 2 µL instead of 5 µL). Changing the protocol or incubation time might have an effect as well, but it is assay specific.

16- Why do I need to change tips when diluting and loading reagents ?

If tips are not changed, it gives false values of lower LDL. Tips MUST be changed while diluting the analyte. It is not required to change tips when loading

the plate if the reagents are always added from the lowest concentration toward the highest concentration.

17-What are the physiological ranges of concentrations of analytes?

Analyte	**Amount in normal human serum(literature)**
EPO	5-25 mIU/mL
Insulin	0-70 µIU/mL
TNFα	1-12 pg/mL
VEGF	13-360 pg/mL

18-Can I use AlphaLISA for diagnostic testing?

Absolutely not. AlphaLISA kits are developed for research purpose only.

19-What is the plate format for AlphaLISA assays?

AlphaLISA can be performed using different plate formats:

- 96-well format; assay volume 50 µl: PerkinElmer ½ AreaPlate-96 recommended (cat. no.6005560, 6005569)
- 384-well format; assay volume 50 µl: all plates performed well, PerkinElmer light-gray AlphaPlate-384 recommended for high counts due to cross-talk reduction (cat. no.6005350, 6005359)
- 384-well format; assay volume 20 µl: PerkinElmer ProxiPlate-384 recommended (cat. no.6008280, 6008289)
- 1536-well format; assay volume 5-10 µl: light-gray AlphaPlate-1536 recommended (cat. no.6004350, 6004359)

20-How many assay points can be done with the kit?

The kits exist in two formats:
500 assay points and 5,000 assay points, based on an assay volume of 50 µl in 96- or 384-well plates using the kit components at the recommended concentrations. By reducing assay volume while maintaining component concentrations, more assay points can easily be performed.

21-How many assay points can be generated in a 1536-well assay format using a small kit or a large kit ?

The number of assay points of the kits and the protocols have been optimized for an assay volume of 50 µl.

Assays can be miniaturized to a 1536- well assay with a final volume of 10 µl, by using proportionally lower reagent volumes and identical final reagent concentration.

As a result, the number of assay points will be increased 5 times, allowing the generation of 2,500 assay points with the small kit and 25,000 with the large kit.

22-What is the shelf life of each kit?

The kits should be stable for at least 12 months from the manufacturing date when stored in their original packaging at the recommended storage conditions.

23-How long are beads stable once they are diluted in buffer?

Long term stability of diluted beads was not evaluated. However, beads will be stable for more than 12 hours once diluted, allowing automated screening and other HTS assays.

24-What is the stability of standard after dilution?

Aliquoted standard is stable for 45 days at -20ºC. Stability at 4ºC will depend on each analyte and was not tested.

25-Does phenol red interfere with the assay?

Phenol red does not interfere with the AlphaLISA signal.

26-Can RPMI media be used?

RPMI medium can be used. However, due to the presence of high level of free biotin, a decrease in total counts and in Signal/Background ratio are expected.

Also, it might affect the Lower Detection Limit (LDL). In most cases, satisfactory results can still be obtained. Other cell culture media which showed to work well are DMEM and MEM.

27-Which instrument is required ?

AlphaLISA can be measured with instruments having an excitation laser, like the PerkinElmer EnVision® Multilabel Plate Reader.

28-Can the assay be automated for HTS?

As no wash steps are required and low sample volumes can be used, it is possible to miniaturize and automate the AlphaLISA assay using the JANUS® Automated Workstation from PerkinElmer.

29-Do DMSO, SDS, EDTA or sodium azide interfere with this assay?

AlphaScreen and AlphaLISA technologies can tolerate DMSO up to 10%, SDS up to 0.02%, EDTA up to 100 mM and sodium azide up to 0.005%. Above these concentrations, signal will decrease, but acceptable results can be obtained. Note that other components of your assay might not tolerate such concentrations (e.g. analyte & protein).

30-Which components are included in the kit and which will be available also as separate items?

Each kit contains:

- biotinylated Ab #1
- Ab #2 coupled to AlphaLISA Acceptor beads
- Streptavidin Donor beads
- Standard (analyte)
- AlphaLISA ImmunoAssay Buffer, 10X

Also available as separate items:

- standard
- AlphaLISA ImmunoAssay Buffer, 10X

Please note that microplates are not included in the kits!

31-Can changes in temperature influence the signal ?

AlphaLISA assays are temperature sensitive because temperature can influence the following two aspects:

- Equilibrium between antibodies and analyte
- Chemical intrinsic reactions (generation and diffusion of singlet oxygen)

Changes as high as 10%/degree Celsius can be observed depending on the assay. Try to keep the temperature as stable as possible.

32-What is the state for multiplexing with AlphaLISA?

At this moment multiplexing in one well is not possible but as the volume of sample needed is so low (typically 5 µL), horizontal multiplexing can be easily performed.

33- PerkinElmer offers an AlphaLISA P24 kit and an ELISA P24 kit. Which should I use?

It depends on your assay requirements:

Requirement	**Assay**
Short Protocol	AlphaLISA
Sensitivity	ELISA
Dynamic Range	AlphaLISA
No Wash Step	AlphaLISA
Small Sample Volume	AlphaLISA
Sample matrix: Cell Culture	AlphaLISA
Sample matrix: Cell culture, Serum & Plasma	ELISA
High throughput	AlphaLISA
Instrumentation	ELISA (any photometric reader) AlphaLISA (requires EnVision with Alpha option)