A Comparison of a Homogenous Bead-Based Amplified Luminescence Technology AlphaLISA and a Colorimetric ELISA for Detection of Human Matrix Metalloproteinase 9 in Complex Sample Matrices

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1 Abstract

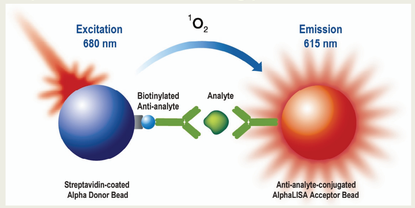
Enzyme-linked Immunosorbent assay (ELISA) is a well known technique in the area of immunoassays. ELISA involves a number of wash steps, which makes it a laborious and time-consuming process. In the field of drug discovery, technologies which can give robust, sensitive, automatable and reproducible immunoassays at a lower cost are highly desirable. Amplified Luminescent Proximity-based Homogeneous Assay (AlphaLISA®) is a bead-based homogenous immunoassay which can give comparable or even better results in less time, cost and sample volume. In this study, we compared ELISA and AlphaLISA platforms commonly used for immunoassays and examined performance (sensitivity, dynamic range, variability) as well as assay complexity and time to perform.

The AlphaLISA matrix metalloproteinase 9 (MMP9) kit, EnVision® Multidetection Plate Reader, and microplates were supplied by PerkinElmer, Inc. The colorimetric ELISA MMP9 kit was from a well-known supplier. The dynamic range, inter- and intra-assay variation was studied in spiked MEM + 10% FBS. Both kits showed good reproducibility and accuracy. Cell culture samples containing MMP9 secreted upon PMA stimulation of U937 cells also showed similar results using the two kits. Using the AlphaLISA assay, we can obtain a PMA dose-response curve of MMP9 induction without any sample dilution, even in RPMI + 10% FBS. The AlphaLISA assay also requires twenty times less sample volume and gives a five-fold wider dynamic range. A wider dynamic range allows the customer to measure most samples without any dilutions. Because the AlphaLISA kit does not require any wash steps, both the elapsed time and hands-on time required to perform the assay was much less than for the ELISA kit.

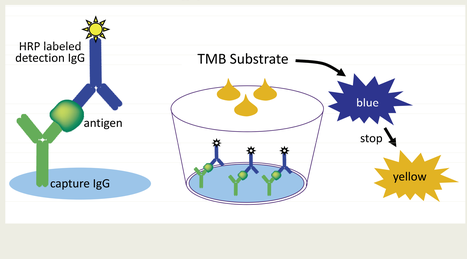
Overall, the AlphaLISA kit offered benefits over the ELISA kit of wider dynamic range, a significantly faster and easier protocol and comparable performance in other respects.

2 Introduction

AlphaLISA Technology



ELISA Technology



3 Materials & Methods

Microplates
AlphaLISA: 96-well ½ area white (PerkinElmer #6005560)
ELISA: Total MMP-9 Microplate (provided with the kit)

Matrix components and compositions for AlphaLISA
- MEM (Invitrogen, #11370021)
- FBS (Hyclone # SH30071, lot # ARF26748)
- For standard curve and unknowns, used MEM + 10% FBS

Matrix components and compositions for ELISA
- For standard curve, supplier recommends diluent provided with kit
- For unknowns, supplier recommends a 100-fold dilution of MEM + 10% FBS

AlphaLISA Kit
AlphaLISA MMP9 Kits (#AL243C, lot P10902)

EnVision® Plate Reader 2104 settings

AlphaLISA
- Distance between plate and detector: 0.15 mm
- Excitation time: 35 ms
- Emission time: 100 ms
- Aperture: 96 Plate HTS AlphaScreen aperture
- Crosstalk correction: no

ELISA
- ELISA Instrument settings
- Instrument: PerkinElmer EnVision
- Absorbance filter 1: 450 nm
- Absorbance filter 2: 540 nm

Protocols

AlphaLISA:
1. Add 5 µL MMP9 standard
2. Add 20 µL mixture of anti-MMP9 acceptor beads and biotinylated anti-MMP9 antibody
3. Incubate 60 minutes at room temperature
4. Add 25 µL streptavidin donor beads
5. Incubate 30 minute at RT in the dark
6. Read on EnVision Reader

ELISA:
1. Add 100 µL of Assay diluent
2. Add 100 µL of MMP9 standard
3. Incubate 120 minutes on horizontal orbital microplate shaker (500±50rpm)

4- 7. Wash plate 4X with 400 µL wash buffer

8. Add 200 µL of conjugate
9. Incubate 60 minutes on horizontal
10. Shake microplate on orbital shaker (500±50rpm)

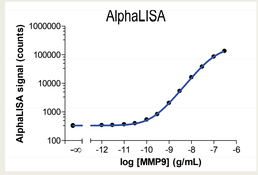
11- 14. Wash plate 4X with 400 µL wash buffer

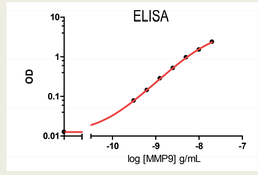
15. Add 200 µL of TMB substrate solution
16. Incubate 30 minutes at RT in the dark
17. Add 50 µL of stop solution
18. Read at 450 nm within 30 minutes (λ correction 540 or 570nm)

Assay conditions for hMMP9 cellular quantitation:

- U937 cells (Suspension cell line ATCC CRL-1593.2)
- Media: RPMI+ 10%FBS
  - RPMI 1640 (Life Technologies, 11835030)
  - FBS (Hyclone Cat # SH30071)
  - PMA (Phorbol 12-Myristate 13 Acetate, SIGMA-ALDRICH, P8139)
- 24 well Tissue Culture Plate (Corning, 3542)
- Number of cells/mL = 3.5 X 10^5
- Volume per well = 1mL

4 Calibration curves





Dynamic range:

Range	AlphaLISA	ELISA
Minimum (LDL)	30 pg/mL	40 pg/mL
Maximum	100,000 pg/mL	20,000 mIU/mL
Total	3.5 Log	2.7 Log

Using their respective and recommended protocols: AlphaLISA and ECL **show similar sensitivities** whilst AlphaLISA has a broader dynamic range.
Sensitivity: Lower detection limit (LDL = mean of background value + 3SD)

5 hMMP9 Recovery

Spiking	Concentrations	AlphaLISA %	ELISA %
% recovery spike-ins	Low	95	100
	Medium	105	90
	High	110	90

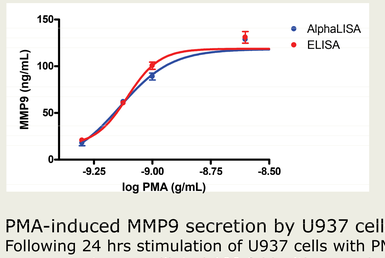
AlphaLISA and ELISA both gave good recoveries.
AlphaLISA spike-in concentrations: 100, 1000 and 10,000 pg/ml
ELISA spike-in concentrations: 250, 1500 and 5000 pg/ml.

6 Assay Precision

Precision		AlphaLISA		ELISA	
		# replicates	%CV	# replicates	%CV
Intra Assay (1 plate)	Low	9	2.3	9	9.1
	Med	9	2.3	9	6.9
	High	9	5.9	9	5.2
Inter Assay (3 distinct plates)	Low	3x9	7.2	3x9	16.9
	Med	3x9	4.6	3x9	6.5
	High	3x9	2.4	3x9	5.4

Compared to ELISA, the AlphaLISA assay allows for better intra- and inter- assay reproducibility.

7 hMMP9 cellular quantitation



PMA-induced MMP9 secretion by U937 cells.
Following 24 hrs stimulation of U937 cells with PMA, assay supernatants were diluted 100 fold with assay buffer and quantified using AlphaLISA and ELISA kits. Both AlphaLISA and ELISA kits **show equivalent results**.

8 Summary

In comparison to ELISA for the detection of hMMP9, the AlphaLISA kit was characterized by:
- Larger assay window (linear dynamic range),
- Similar sensitivity (based on LDL),
- Better intra- and inter-assay precision (lower %CV)
- Equivalent performance in cell based assays.

The AlphaLISA assay had these advantages over ELISA:
- Lower sample volume requirement for equivalent performance
- Shorter total assay duration
- No wash steps
- No shaking
- No need for special plates

This comparative study demonstrates the excellent performance and markedly enhanced and ease of use of the AlphaLISA technology over ELISA.

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