APPLICATION NOTE

# Detection of human matrix metalloproteinase 9 in complex sample matrices.

## A comparison of AlphaLISA and colorimetric ELISA immunoassays


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### Introduction


While ELISA (enzyme-linked immunosorbent assay) technology has been considered the gold standard of immunoassays, it 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. The AlphaLISA™ assay (amplified luminescent proximity-based homogeneous assay) is a bead-based homogeneous immunoassay which can give comparable or even better results in less time, cost and sample volume. In this study, we compared the ELISA and AlphaLISA immunoassay platforms in two complex sample matrices (serum-containing culture media and in cell supernatants). We examined key performance parameters (sensitivity, dynamic range, variability) as well as assay complexity and time to perform. 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.




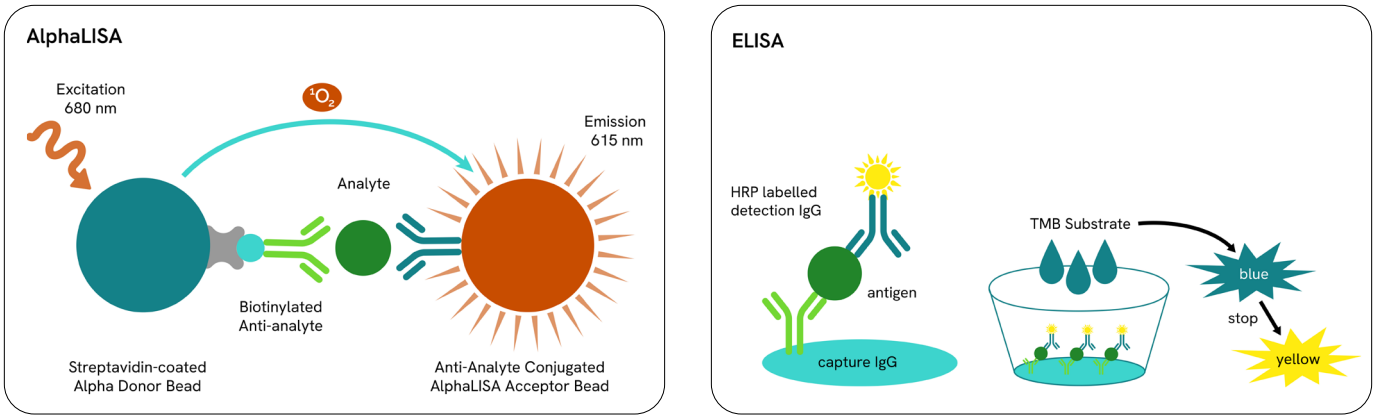


Figure 1: **Comparison of AlphaLISA and ELISA immunoassay configurations.**
Left panel: For AlphaLISA, epitope-specific antibodies are either biotinylated or directly conjugated on to the Acceptor beads. In the presence of the analyte of interest, Streptavidin-Donor and Acceptor beads are brought into proximity allowing the AlphaLISA signal to be generated following laser excitation at 680 nm.
Right panel: For ELISA, a capture IgG specific to the antigen of interest is coupled to the microplate whereas another IgG is labeled with horseradish peroxidase (HRP, detection IgG). After a series of blocking and wash steps to remove excess detection IgG non-specifically bound to the antigen, TMB (a blue colored substrate for HRP) is added. Upon reaction of TMB and HRP, a yellow colored product is generated which is proportional to the amount of HRP detection IgG bound to the antigen of interest.

## Materials and methods

### Materials

The AlphaLISA MMP-9 kit ((#AL243C), EnVision™ Multilabel Reader, and 96-well half area white microplates (#6005560) were supplied by Revvity. The colorimetric ELISA MMP-9 kit and microplates were obtained from a well-known kit provider (Supplier R). The dynamic range, inter- and intra-assay variation was studied using MMP-9 analyte spiked into MEM + 10% FBS.

AlphaLISA samples and standards were prepared in a complex matrix consisting of minimal essential media (MEM (Invitrogen, #11370021) + 10% heat-inactivated FBS (Wisent, #080450).

ELISA samples were prepared in MEM + 10% heat-inactivated FBS and diluted 100-fold in the diluent provided, according to the kit manufacturer's recommendations. Standards were also diluted in the diluents provided.

### Assays of stimulated cell extracts

Cell-based MMP-9 assays were performed using U937 suspension cells (ATCC, #CRL-1593.2) cultured in RPMI + 10% FBS. Cells were grown in 24 well tissue culture plates (Corning, #3542) to a density of 3.5 X $10^5$ cells/mL (1 mL/well) and stimulated by addition of PMA (phorbol 12-myristate 13 acetate) for 24 hours.

The suppliers for media components were: RPMI 1640 (Invitrogen, #11835030, FBS (Hyclone, #SH30071), PMA (Sigma, #P8139).

## Results and discussion

To compare the lower limit of detection and dynamic range of the AlphaLISA and ELISA assays, MMP-9 calibration curves were generated using the protocols recommended by the manufacturers. Lower detection limit (LDL) values were calculated as the mean of background value + 3SD. The AlphaLISA and ELISA assays showed similar sensitivities for detection of MMP-9 in MEM + 10% FBS. The dynamic range for AlphaLISA was 30-100,000 pg/mL and 40-20,000 pg/mL for ELISA. Overall, the AlphaLISA assay has a considerably wider dynamic range.

Table 1: EnVision Multilabel Reader settings

| AlphaLISA | ELISA (colorimetric) |
|---|---|
| **Distance between plate and detector: 0.15 mm** | **Absorbance filter 1: 450 nm** |
| **Excitation time: 35 ms** | **Absorbance filter 2: 540 nm** |
| **Emission time: 100 ms** | |
| **Aperture: 96 Plate HTS AlphaScreen™ aperture** | |
| **Crosstalk correction: No** | |

Table 2: Comparison of immunoassay protocols

| Step | AlphaLISA assay protocol | ELISA assay protocol |
|---|---|---|
| **1** | Add 5 µL MMP-9 standards or samples. | Add 100 µL of assay diluent. |
| **2** | Add 20 µL mixture of anti-MMP-9 Acceptor beads and biotinylated anti-MMP-9 antibody. | Add 100 µL of MMP-9 standards. |
| **3** | Incubate 60 minutes at room temperature. | Incubate 120 minutes on orbital microplate shaker (500 ± 50 rpm). |
| **4** | Add 25 µL streptavidin donor beads. | Wash plate 4X. |
| **5** | Incubate 30 minutes at room temperature in the dark. | Add 200 µL enzyme-antibody conjugate. |
| **6** | Read plate on EnVision™ Multilabel Plate Reader. | Incubate 60 minutes on orbital microplate shaker (500 ± 50 rpm). |
| **7** | | Wash plate 4X. |
| **8** | | Add 200 µL TMB substrate solution. |
| **9** | | Incubate 30 minutes at room temperature in the dark. |
| **10** | | Add 50 µL stop solution. |
| **11** | | Read on EnVision Multilabel Plate Reader at 450 nm within 30 minutes (λ correction 540 or 570 nm). |
| | **2.5 hours overall<br>0 washes** | **5-6 hours overall<br>8 washes** |

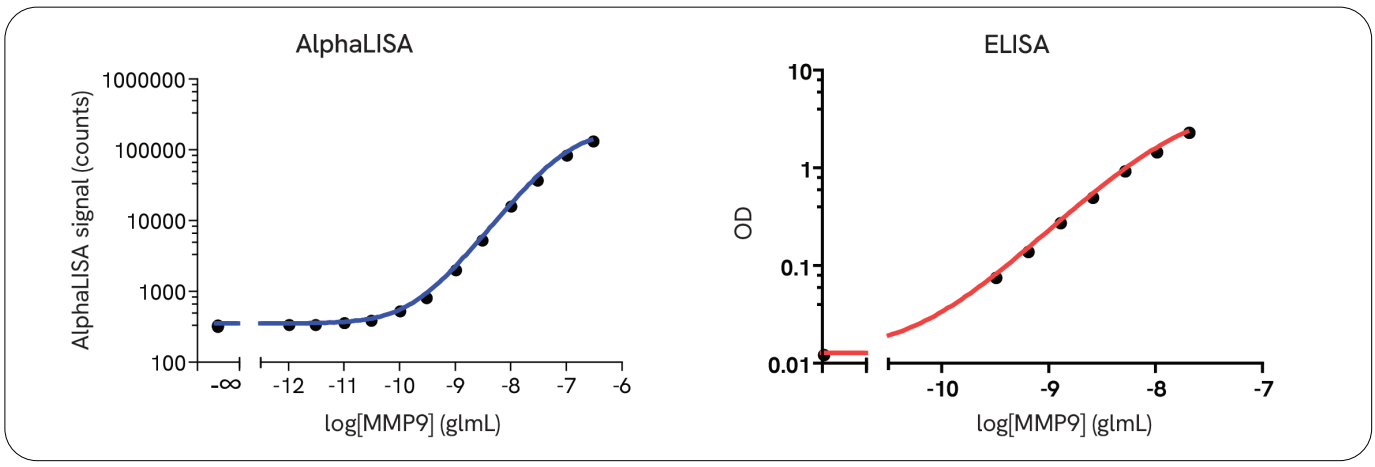


Figure 2: MMP-9 AlphaLISA (left panel) and ELISA (right panel) calibration curves in sample matrix containing 10% FBS.

The accuracy of MMP-9 detection was then assessed using three different analyte concentrations spanning the linear part of the MMP-9 standard curves. The low, medium and high MMP-9 spike-in concentrations were 100, 1000 and 10,000 pg/mL for AlphaLISA; and 250, 1500 and 5000 pg/mL for ELISA. When tested with their own analyte standards, the AlphaLISA and ELISA kits showed suitable percent recovery values (Table 3).

**Intra- and inter-assay precision**

The Intra-assay precision was determined using 9 replicates for each of the 3 concentrations of MMP-9 used above to determine percent recovery. Inter-assay precision was determined using the same protocol repeated during 3 independent experiments. As shown in Table 4, the AlphaLISA assay provides better intra- and inter- assay reproducibility.

Table 3: Accuracy of MMP-9 detection in MEM + 10% FBS.

| Spiked-in MMP-9 concentration | AlphaLISA % recovery | ELISA % recovery |
|---|---|---|
| Low | 95 | 100 |
| Med | 105 | 90 |
| High | 110 | 90 |

Table 4: MMP-9 intra- and inter- assay precision

| Precision | Spiked-in MMP-9 concentration | AlphaLISA | | ELISA | |
|---|---|---|---|---|---|
| | | # Replicates | %CV | # Replicates | %CV |
| Intra-assay | Low | 9 | 2.3 | 9 | 9.1 |
| | Med | 9 | 2.3 | 9 | 6.9 |
| | High | 9 | 5.9 | 9 | 5.2 |
| Inter-assay | Low | 3x9 | 7.2 | 3x9 | 16.9 |
| | Med | 3x9 | 4.6 | 3x9 | 6.5 |
| | High | 3x9 | 2.4 | 3x9 | 5.4 |

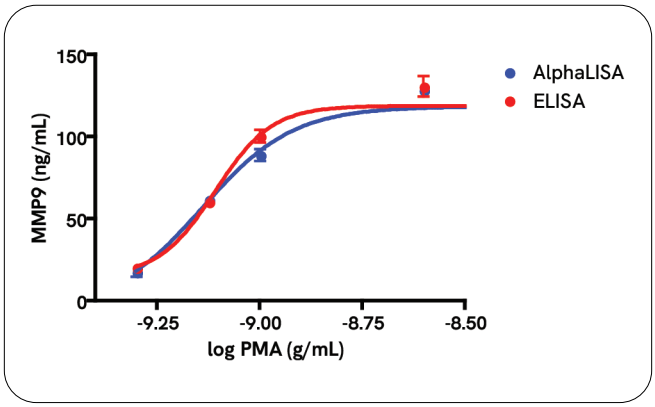


Figure 3: Dose dependence of MMP-9 release by U937 cells in response to PMA stimulation.

**MMP-9 assays in PMA-stimulated cells**

These assay platforms were next compared for the measurement of MMP-9 released by U937 cells upon stimulation by PMA. Following 24 hrs stimulation of U937 cells with 0.5-5 pg/mL of PMA, assay supernatants were diluted 100-fold with respective assay buffer and quantified using the AlphaLISA and ELISA kits. As shown in Figure 3, the AlphaLISA and ELISA kits showed similar levels of MMP-9 release in response to PMA stimulation.

## Summary

The current comparison between AlphaLISA and ELISA revealed that both kits deliver good sensitivity and accuracy. However, the AlphaLISA assay demonstrated better intra- and inter-assay reproducibility compared to the ELISA assay. U937 cells secreting MMP-9 in response to PMA stimulation also showed similar results using the two kits. Using AlphaLISA, we can obtain a PMA dose-response curve of MMP-9 induction without any sample dilution (data not shown), even in RPMI + 10% FBS. AlphaLISA 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 the benefits of a significantly faster and easier protocol, while offering equal or better performance in other respects. These process benefits, combined with the generally lower cost of AlphaLISA reagents make the AlphaLISA assay platform particularly attractive for high throughput screening applications.

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