

# Homogeneous IgG AlphaLISA<sup>®</sup> assay performed on BMG LABTECH's PHERAstar Plus

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- IgG AlphaLISA<sup>®</sup> assay performed on BMG LABTECH's PHERAstar Plus
- Z' value of 0.92 indicates a highly robust assay combined with high quality instrumentation
- IgG limit of detection determined to be < 0.21 ng/mL

## Introduction

Immunoglobulins or antibodies are proteins that are involved in the immune response. They bind with high affinity at exogenous substances (antigens). Immunoglobulins (Ig) are classified according to the structure. The most present antibody in plasma is IgG. It is part of the secondary antibody response and is built 3 weeks after antigen recognition. There are several assays for the determination of IgG commercially available. This application note shows the detection of IgG using an AlphaLISA<sup>®</sup> assay performed on BMG LABTECH's PHERAstar Plus.

## Assay Principle

The AlphaLISA<sup>®</sup> technology from PerkinElmer is based on the AlphaScreen<sup>®</sup> detection method: Upon laser excitation at 680 nm of Donor beads ambient oxygen is converted into singlet oxygen released at a rate of up to 60,000 molecules per second. Singlet oxygen molecules have a short lifetime (4  $\mu$ s in aqueous solutions) and diffuse of no more than 200 nm. When a biomolecular interaction brings the Donor and Acceptor beads in proximity, the singlet oxygen reaches the Acceptor bead and a cascade of chemical reactions is initiated producing a greatly amplified luminescence signal. The assay principle of the IgG AlphaLISA<sup>®</sup> assay is given in Figure 1.

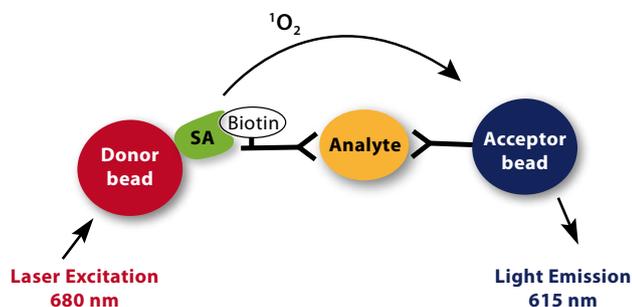


Fig. 1: Principle of the IgG (Analyte) AlphaLISA<sup>®</sup> assay kit

Anti-IgG antibody coated Acceptor beads and biotinylated anti-IgG antibody bind to IgG. In a second step streptavidin-coated Donor beads are added and bind to the biotinylated anti-IgG antibody. Donor and Acceptor beads are in close proximity when IgG is present in the sample. After laser excitation at 680 nm a cascade of chemical reactions is started resulting in a luminescence signal at 615 nm. If no IgG is present, no light emission is detected.

## Materials and Methods

- AlphaLISA<sup>®</sup> IgG Kit, PerkinElmer #AL205C
- White opaque 384-well OptiPlate<sup>™</sup>, PerkinElmer #6007290
- White 384-well small volume plate, Greiner #784075
- PHERAstar Plus microplate reader, BMG LABTECH, Offenburg, Germany, Figure 2



Fig. 2: BMG LABTECH's PHERAstar Plus microplate reader

The IgG AlphaLISA<sup>®</sup> standard curve was performed in accordance with the kit protocol. Briefly, a serial dilution of lyophilized IgG was prepared in AlphaLISA<sup>®</sup> immunoassay buffer. A mix of Acceptor beads and biotinylated antibody were added to each of the 12 standards and to the blank (contains no IgG). Following 60 min incubation at room temperature, Donor beads were added. As Donor beads are light sensitive this step has to be done under subdued or green light. After another 30 min of incubation in the dark the AlphaLISA<sup>®</sup> signal is measured in the PHERAstar Plus. BMG LABTECH has developed an AlphaLISA<sup>®</sup> specific optic module and the instrument settings for a 384-well plate can be found below.

### Instrument Settings

Measurement method	AlphaScreen <sup>®</sup>
Reading mode	Endpoint
Optic module	AlphaLISA <sup>®</sup> 680 615

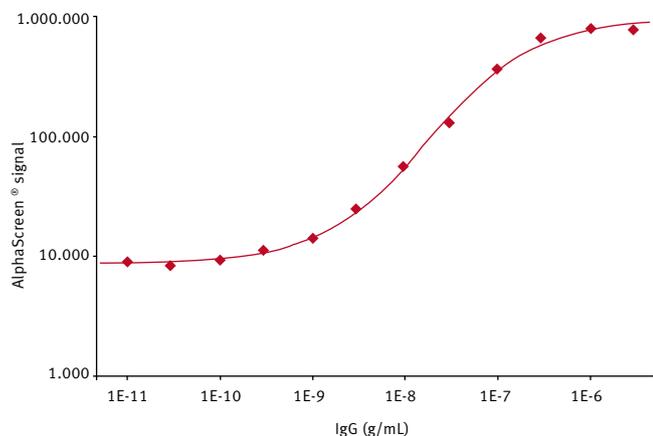
### General Settings

Positioning delay	0.10 s
Excitation time	0.30 s
Integration start	0.34 s
Integration time	0.30 s
Gain	3600

850  $\mu$ L of each standard and blank were prepared in vials. After incubation a certain amount of reaction mix was transferred into the two different types of 384-well microplates. 50  $\mu$ L were used for the 384-well standard plate and 17  $\mu$ L were the final volume in the 384-well small volume plate. Each plate consisted of 12 replicates for both the standards containing different IgG concentrations and the blank. Because of the different fill heights it is recommended to perform a focus adjustment once for every plate type. The focal height will be optimized resulting in the highest possible sensitivity.

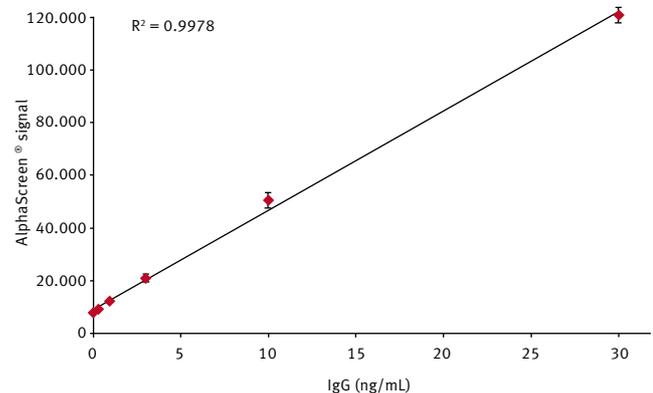
## Results and Discussion

Figure 3 shows an IgG standard curve obtained in 384-well plates using a volume of 50  $\mu$ L.



**Fig. 3:** A typical log-log IgG standard curve recorded on the PHERAstar Plus using the AlphaLISA<sup>®</sup> specific optic module in 384-well format

For low concentrations the linear - linear scale is preferred (Figure 4). With the MARS data analysis software both standard curves can easily be plotted.



**Fig. 4:** A linear-linear chart at low IgG concentrations obtained with the MARS data and analysis software.

Z' values, a standard in evaluating HTS methods, and the limit of IgG detection were calculated for both plate types (Table 1). The formulas for both calculations are given below.

$$Z' = 1 - \frac{3\sigma_p + 3\sigma_n}{|\mu_p - \mu_n|}$$

where  $\mu_p$  = mean of "positive control" (max ratio),  $\mu_n$  = mean of "negative control" (min ratio), and  $\sigma$  = the corresponding standard deviations.

The limit of detection (LOD) was determined by interpolating the average blank values + 3x the standard deviation of the blank on the standard curve (4-parameter fit).

**Table 1:** Z' values and LOD of IgG in 50  $\mu$ L and in 17  $\mu$ L

Plate Format	384-well standard plate 50 $\mu$ L	384-well small volume plate 17 $\mu$ L
Z' value	0.92	0.91
Limit of IgG detection (ng/mL)	0.21	0.27

## Conclusion

The IgG AlphaLISA<sup>®</sup> detection kit was successfully performed on the PHERAstar Plus. The sensitivity stated by the kit manufacturer was reached in 384-well standard plates and was slightly better than in 384-well small volume format. Nonetheless, we could show that it is possible to decrease the assay volume to 17  $\mu$ L with good sensitivity and very good Z' values.

The multidetection HTS reader PHERAstar Plus shows great performance in AlphaScreen<sup>®</sup> and AlphaLISA<sup>®</sup> mode in 384-well small volume plate format. The easy to use software allows simple assay optimization regarding sensitivity and read times. The PHERAstar Plus has been designed to read all HTS detection modes (fluorescence intensity, timeresolved fluorescence, fluorescence polarization, luminescence, AlphaScreen<sup>®</sup>, and absorbance) in all plate formats up to 1536 wells.

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