Miniaturization of a Cell-based TNF-α AlphaLISA® Assay Using Echo® Liquid Handler and the PHERAstar *FS*

Carl Peters[1] and Bonnie Edwards[2] [1]BMG LABTECH, Cary, NC, USA [2]Labcyte, Sunnyvale, CA, USA

Application Note 260, Rev. 12/2014

- AlphaLISA assay can be miniaturized down to 2 µl in 1536-well microplates
- TNF-α was detected in stimulated THP-1 cells and antagonist potency was determined

Introduction

Tumor necrosis factor (TNF)-α is mainly secreted by macrophages and monocytes where it plays an important role in several functions of the immune system, such as inflammation and cell death. However, overproduction of TNF-α has been linked to inflammatory disorders such as rheumatoid arthritis.[1] Therefore, screening for potential inhibitors of TNF-α production could lead to beneficial treatments for inflammatory diseases.

Miniaturization for screening purposes presents an attractive scenario in which drug discovery can take place while saving on expenditure of target compounds and detection reagents. For assay miniaturization to be successful liquids must be handled with extreme accuracy and the small volumes require highly sensitive detection. Here we describe the use of the PHERAstar *FS* from BMG LABTECH to detect an AlphaLISA® assay which has been miniaturized by the Echo® Liquid Handler from Labcyte.

Assay Principle

The TNF-α AlphaLISA® Immunoassay uses antibodies specific for the analyte (TNF-α) that are coupled to donor and acceptor beads. If the analyte is present both donor and acceptor beads will bind via their antibodies to the analyte. That brings donor and acceptor beads into proximity such that an emission signal at 615 nm can be detected following laser excitation at 680 nm.

The Echo® Liquid Handler from Labcyte employs sound energy to transfer liquids in nanoliter volume increments with precision and accuracy. The transfer is tipless, eliminating the risk of carryover while offering additional cost savings.

Materials and Methods

- ☐ AlphaLISA® Human TNF-α Research Immunoassay Kit (PerkinElmer, #AL208C)
- ☐ THP-1 cells (ATCC, #TIB-202)
- ☐ (-)-Isoproterenol hydrochloride, salbutamol, histamine dihydrochloride and lipopolysaccharides (LPS) were all obtained from Sigma
- ☐ Echo 555 Liquid Handler and 384-well Echo qualified source microplates (Labcyte)
- ☐ 1536-well white, solid-bottom assay microplate (Labcyte)
- ☐ PHERAstar *FS* microplate reader (BMG LABTECH, Fig. 1)

Fig. 1: PHERAstar *FS* microplate reader from BMG LABTECH

Laser excitation and assay specific optic modules enhance performance of AlphaLISA.

For all experiments liquid was added using the Echo® Liquid Handler unless otherwise noted. After every transfer step a brief centrifugation was done (1 sec/1000 rpm) before the plate was sealed or lidded.

TNFα standard dilution series

Initial miniaturization experiments were performed to ensure the ability to detect known amounts of analyte. To accomplish this 1.6 µl of analyte was added followed by 200 nl of a mixture containing acceptor beads and anti-TNF-α antibody. Following a one hour incubation at room temperature in the dark 200 nl of donor beads was added for a final volume of 2 µl. After an additional 30 minute room temperature incubation in the dark plates were read on the PHERAstar *FS*.

TNFα production in THP-1 cells

Subsequent experiments sought to assess the ability to detect TNF-α production by THP-1 cells and the effect of various compounds on the production of TNF-α. First 5 nl of compound was transferred to plates. Then 3000 cells were dispensed in 1.5 µl using a Multidrop™ Combi followed by addition of 95 nl of LPS. Following a three hour incubation at 37°C 200 nl of acceptor beads/ anti-TNF-α antibody was added. After a one hour incubation at room temperature 200 nl of donor beads was added for a final volume of 2 µl. After an additional 30 minute room temperature incubation plates were read on the PHERAstar *FS*.

For both experiments plates were read using the following settings:

Measurement Type:	AlphaScreen®
Reading Mode:	Endpoint
Optic Module:	AlphaLISA®
Excitation wavelength:	680 nm
Emission wavelength:	615 nm
Positioning delay (sec):	0.1
Excitation time (sec):	0.30
Integration start (sec):	0.34
Integration time (sec):	0.60
Gain:	3600

Results and Discussion

Several dilutions of TNF-α were tested to confirm the ability of the Echo liquid handler to accurately dispense the AlphaLISA reagents and the PHERAstar *FS* to sensitively detect a 2 µl assay (Figure 2).

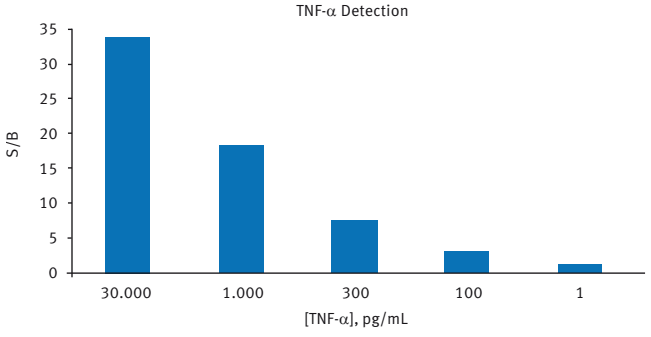

Fig. 2: TNF-α detection in a miniaturized assay

The results in Fig. 2 indicate that in an assay volume of as little as 2 µl TNF-α was detected across a range of concentrations with high signal to background and sensitivity.
Fig. 3 shows the high linearity that is obtained at low analyte concentrations.

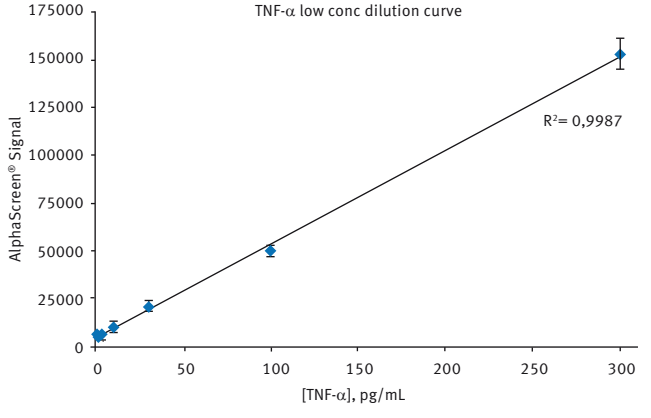

Fig. 3: Linear relationship of low TNF-α concentration and AlphaScreen® /AlphaLISA® signal. Error bars refer to triplicates.

Because of the use of donor and acceptor beads in Alpha technology it was investigated whether over time there was any variability in this AlphaLISA assay. No significant variability was seen after 1 hour indicating that settling of beads in the source plate was not a problem (Data not shown).

THP-1 cells are a human monocytic cell line well characterized for their ability to produce TNF-α in response to treatment with LPS. Previous results have indicated that beta adrenergic receptor and histamine H2 receptor agonists have an inhibitory effect on LPS induction of TNF-α by monocytes.[2,3]

The results of this miniaturized assay correspond to those predicted (Figure 4).

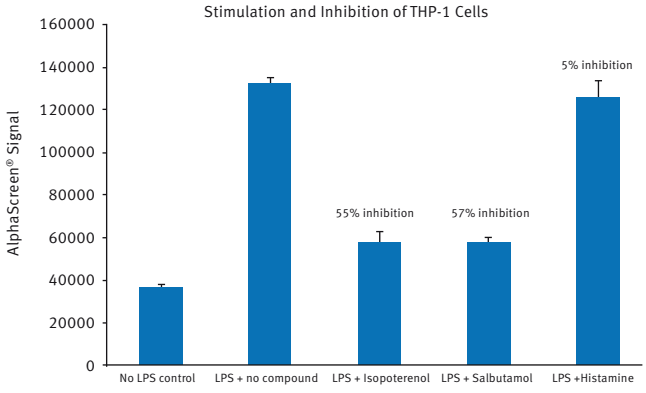

Fig. 4: TNF-α production in THP-1 cells. Cells were stimulated with LPS and inhibition effect is presented for different compounds.

Treatment with LPS exhibits a greater than 3 fold increase in signal while co-treatment with the beta adrenergic receptor agonists leads to greater than 50% inhibition of this increase. Treatment with histamine had a much more modest effect (5% inhibition).

Conclusion

The TNF-α AlphaLISA assay has been successfully miniaturized using Labcyte and BMG LABTECH technologies. The ability to miniaturize these assays and thus reduce reagent use by up to 25 fold, can provide significant cost savings, as well as reduction in compound and sample/cell usage.

References

1. Blandizzi, C et al. The role of tumor necrosis factor in the pathogenesis of immune-mediated diseases. Int. J. Immunopathol. Pharmacol. (2014) **27**: 1-10.
2. Severn, A et al. Regulation of tumor necrosis factor production by adrenaline and beta-adrenergic agonistis. J. Immunol. (1992) **148**: 3441-3445.
3. Morichika, T et al. Histamine inhibits lipopolysaccharide-induced tumor necrosis factor-alpha production in an intracellular adhesion molecule-1- and B7.1-dependent manner. J Pharmacol. Exp. Ther. (2003) **304**: 624-633.

Germany:	**BMG LABTECH GmbH**	**Tel: +49 781 96968-0**
Australia:	BMG LABTECH Pty. Ltd.	Tel: +61 3 59734744
France:	BMG LABTECH SARL	Tel: +33 1 48 86 20 20
Japan:	BMG LABTECH JAPAN Ltd.	Tel: +81 48 647 7217
UK:	BMG LABTECH Ltd.	Tel: +44 1296 336650
USA:	BMG LABTECH Inc.	Tel: +1 877 264 5227
Internet:	**www.bmglabtech.com**	**applications@bmglabtech.com**