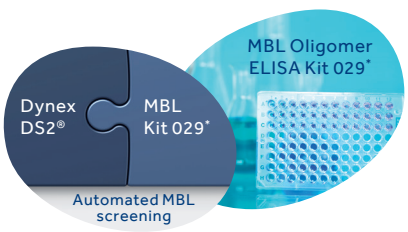
PERFORMANCE DATA
AND APPLICATION NOTE FOR

MBL Oligomer ELISA Kit 029

Dynex Technologies DS2



Version: 01
Revision: MO2017-08-EN

Objectives
The paper is an assessment of levels of MBL in human serum samples using Bioporto Diagnostics MBL Oligomer ELISA Kit 029 on the Dynex Technologies DS2 platform.

Introduction
Mannan-binding lectin (MBL) is an important component of the innate immune system. However, in at least 12% of the average Caucasian population, the circulating level of functional MBL is insufficient. This makes MBL deficiency by far the most common primary immunodeficiency. MBL is a plasma protein that activates the complement system on binding to invading pathogens including bacteria, viruses, protozoa and fungi. This leads to the phagocytosis or lysis of the invading microorganisms. MBL also has more subtle immunomodulatory effects. Only the normally oligomerized forms of MBL are functional, i.e. capable of binding efficiently to microbial carbohydrates.

The assay is an ELISA performed in microwells coated with a monoclonal antibody against the MBL carbohydrate-binding domain. Bound MBL is detected with the same antibody that has been labelled with biotin, followed by development with horseradish peroxidase (HRP)-conjucted streptavidin and incubation with a chromogenic substrate. Comparison of the assay results with molecular size chromatography of MBL immunoreactivity in individual human serum samples suggests that the monoclonal antibody used is selective for MBL oligomers when used as both capture and detection antibody.

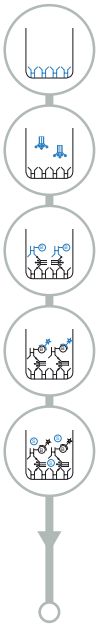
Procedure
The assay is a four-step procedure:

Step 1. Aliquots of calibrators, diluted serum samples and any controls are incubated in micro wells pre-coated with monoclonal antibody against MBL. MBL present in the solutions will bind to the antibody-coated wells via its carbohydrate-binding domains. Unbound material is removed by washing.

Step 2. Biotinylated monoclonal detection antibody is added to each test well and incubated. The detection antibody attached to bound MBL oligomers via carbohydrate-binding domains that are not occupied by being bound down to the coat. Unbound detection antibody is removed by washing.

Step 3. HRP-conjugated streptavidin is added to each test well and allowed to from a complex with the bound biotinylated antibody. Unbound conjugate is removed by washing.

Step 4. A chromogenic peroxidase substrate containing tetramethylbenzidine (TMB) is added to each test well. The bound HRP-streptavidin reacts with the substrate to generate a coloured product. The enzymatic reaction is stopped chemically, and the colour intensity is read at 450 nm in an ELISA reader. The colour intensity (optical density) is a function of the concentration of MBL oligomeric forms originally added to each well. The results for the calibrators are used to construct a calibration curve from which the concentrations of MBL in the test specimens are read.



MBL antibody
Plates are precoated with MBL antibody. The plates are ready for use

MBL
Diluted samples and calibrators are added to each well and incubated

Biotinylated MBL Antibody
Biotinylated detection antibody is added to each well and incubated

Streptavidin – HRP
HRP-conjugated streptavidin is added to each well and incubated

TMB Substrate
Substrate is added to each well. Develop for 10 minutes in the dark

Stop Solution
Stop Solution is added to each well. Read plate within 30 min.

Quantitative results are obtained by measuring the absorbances of the wells at 450 nm

REF	Product Name
Kit 029	MBL Oligomer ELISA Kit

Number of determinations:
1 Kit provides determination of 40 samples in duplicate and a standard curve.

To use BioPorto's MBL Oligomer ELISA Kit on the Dynex Technologies DS2 chemistry analyser the reagents must first be transferred into the appropriate containers supplied with the machine.

(!) Read the instructions for Dynex Technologies DS2 before transferring the reagents.

NOTE
Only transfer the amount of reagents needed for the test. For further information read Bioporto's IFU.

PERFORMANCE DATA
The performance data shown were obtained by BioPorto A/S. For additional performance data and product application, please read the instructions for use accompanying the products carefully. Each individual laboratory should validate the use of MBL Oligomer ELISA Kit on its system.

LIMIT OF DETECTION (LoD)
The detection limit is determined to 5 pg/mL

RANGE
The measuring range of MBL Oligomer ELISA Kit 029 is 0-40 ng/mL

CALIBRATOR RECOVERY AND ANALYTICAL RECOVERY:
Analytical recovery of calibrator material is within acceptance limits.
The recovery of QC sample dilutions 1-2 spiked with different amounts of MBL gave recoveries of 101% and 102%.

Serum sample	Analytical recovery (mean of 5 dilutions)
1	102%
2	101%

INTRA-ASSAY VARIATION
CV`s of QC sample recoveries are from 3,8%-5,5%.

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