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SARS-CoV-2 research

A rapid, fully automatable bead-based SARS-CoV-2 ELISA in only 45 minutes

Keywords

Dynabeads, ELISA, SARS-CoV-2, trimeric spike protein, IgG, IgM, total Ig, coronavirus, KingFisher, Flex, Apex, automation

In this application note, we show:

- A rapid 45-minute ELISA based on Dynabeads magnetic beads for detection of SARS-CoV-2 antibodies from human serum or plasma
- Simple, fast, and reliable manual and automated magnetic bead–based methods for quantitative and qualitative ELISAs for SARS-CoV-2 research

Introduction

Detection of SARS-CoV-2 antibodies is used to confirm if an individual has been exposed to and generated an immune response against the virus. The enzyme-linked immunosorbent assay (ELISA) technique is utilized to identify specific immunoglobulins, quantify the immune response, and help stratify the sera or plasma from infected individuals for further downstream research. The gold-standard detection method for research use is a plate-based ELISA where SARS-CoV-2 antigens are conjugated to the surface of the ELISA plate wells. The antigens capture antibodies present in the added sample, and then an antibody conjugated to a reporter such as horseradish peroxidase (HRP) generates a signal for detection. This manual plate-based ELISA often takes several hours to perform, due to the slow binding kinetics, many washing steps, and a lot of hands-on work.

An automated bead-based ELISA method yielding the expected sensitivity, recovery, and dynamic range with a faster, more reproducible workflow and less hands-on time versus standard plate-based ELISAs is highly desirable in SARS-CoV-2 research.

Fast and simple automated SARS-CoV-2 antibody detection

Here we describe a short, simple, bead-based, and automated ELISA approach that reduces potential user errors, increases throughput, and frees up time for research priorities. Based on Invitrogen™ Dynabeads™ magnetic beads, this new ELISA method significantly reduces total assay time and facilitates automated ELISA using Thermo Scientific™ KingFisher™ instruments to obtain higher throughputs with less hands-on time. By conjugating a trimeric SARS-CoV-2 spike protein to the Dynabeads magnetic beads, we take advantage of the built-in properties of these monosized, monodisperse superparamagnetic beads, which include low nonspecific binding, high reproducibility, and fast binding kinetics. Fast binding kinetics significantly reduce both the incubation times and overall assay time (Figure 1).

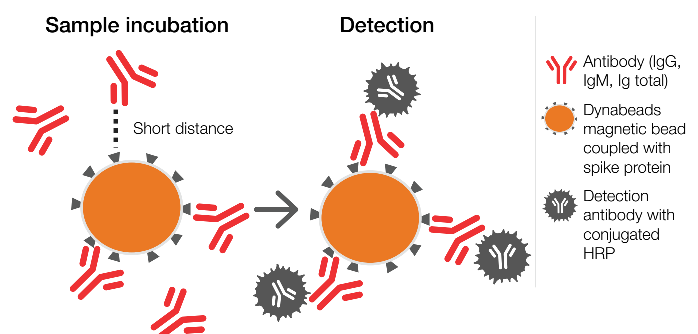


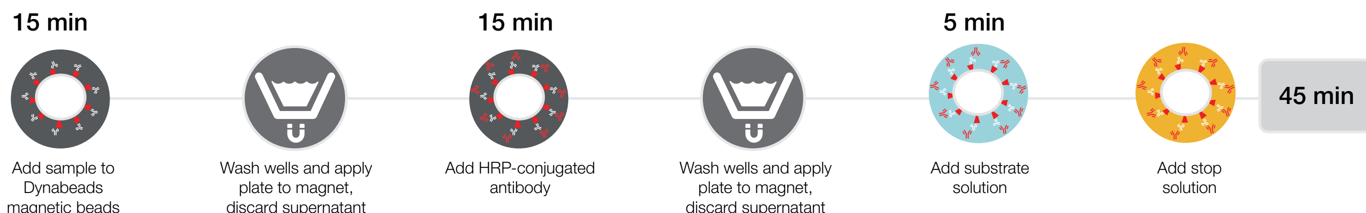
Figure 1. Binding kinetics. Due to the relatively short distance between target and bead, the constant mobility, and the concentration of the trimeric spike protein conjugated on the bead surface, the Dynabeads magnetic bead–based approach is able to reduce incubation times and produce repeatable results in SARS-CoV-2 research.

Rapid bead-based ELISA for SARS-CoV-2 antibody quantification with a manual or automated workflow

To estimate the concentration of total Ig, IgG, and IgM antibodies against SARS-CoV-2 in serum samples, we have developed a rapid bead-based ELISA method with an assay time of only 45 minutes (Figure 2). The assay can be performed manually (Figure 2A) or automated on a 96-plate Thermo Scientific™ KingFisher™ Flex or Apex instrument (Figure 2B). The spike protein–coated Dynabeads magnetic beads can also be used in a newly developed flow cytometry assay for estimating the right dilution factor required for quantitative ELISA from an unknown sample (see application note COL26503 for details). For a quantitative manual bead-based ELISA (Figure 2A), 30 µL of Dynabeads magnetic beads (1 mg/mL) were loaded per well in white, nontransparent U-bottom ELISA plates along with 70 µL of assay buffer, for a total volume of 100 µL. A 96-well plate magnet was used to collect the beads. The buffer was removed by inverting the plate while it was still on the magnet, before adding 100 µL of prediluted sample. The sample was incubated for 15 minutes at 37°C with shaking.

After incubation, the wells were washed 3 times with 200 µL wash buffer. For target detection, 100 µL of detection antibody was added, followed by 15 minutes of incubation at 37°C with shaking. After a second round of washing (3 times with 200 µL wash buffer), 100 µL of substrate was added and incubated in the dark at 37°C for 5 minutes with shaking. During all incubation steps, the plates were covered with plastic film to prevent cross-contamination. The reaction was stopped by adding 100 µL of stop solution, and the plate was put on a 96-well plate magnet. A portion of each well's contents (100 µL) was transferred to a flat-bottom ELISA plate, and absorbance was measured at 450 nm. For ease of use (Figure 2B), the manual bead-based ELISA method can be transferred to the KingFisher instrument, offering a fast walk-away solution. The hands-on activities are limited to the plate loading according to the automated protocol and the addition of stop solution as the final step of the protocol.

A Manual



B Automated

Figure 2. Manual (A) and automated (B) ELISA workflows based on Dynabeads magnetic beads coated with SARS-CoV-2 spike protein. The beads can be purchased separately or as part of our Invitrogen™ Dynabeads™ SARS-CoV-2 Spike immunoglobulin ELISA kits.

Dynamic range

The dynamic range of the assay spans the lowest to highest values of target that the assay can measure with acceptable precision and accuracy. Dynamic ranges were measured using the standard curves for SARS-CoV-2 total Ig, SARS-CoV-2 IgG, and SARS-CoV-2 IgM (Figure 3). Eight-point standard curves were prepared in assay buffer and were therefore independent of the sample matrix. The standard curves were generated using the Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader and Thermo Scientific™ SkanIt™ Software, and analyzed using 4-parameter logistic (4PL) regression. All standard curves showed good model curve fits with $R^2 > 0.999$. For all 3 targets, there was high correlation between the amount of target added to a serum sample and the amount of target measured (recovery), over the full range of target concentrations (blue points in Figure 3). Similar performance was obtained in EDTA plasma, citrate plasma, and heparin plasma (data not shown).

Precision

Intra-assay precision (repeatability) was assessed from data obtained by one operator analyzing 6 replicate samples from one serum batch spiked with different concentrations of IgG, total Ig, and IgM (Figure 4). Inter-assay precision (intermediate precision) was assessed from data obtained by 3 operators over 3 different days using 3 serum samples that contained different concentrations of IgG, total Ig, and IgM. Different concentrations covering the linear range of the standard curve were tested, but only one concentration is shown for each target. Both the intra-assay and inter-assay precision were high. Similar performance was obtained in EDTA plasma, citrate plasma, and heparin plasma (data not shown).

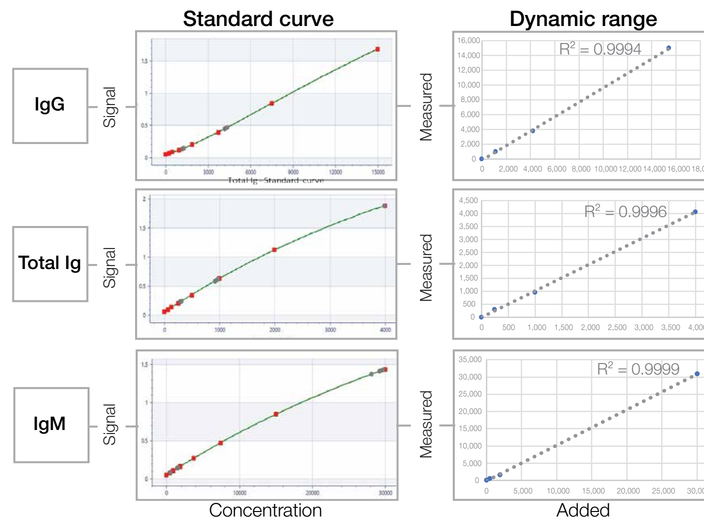


Figure 3. Dynamic range. Standard curves were generated for IgG, total Ig, and IgM, using the Varioskan LUX Multimode Microplate Reader and SkanIt Software, and analyzed by 4PL regression. All standard curves showed good model curve fits with $R^2 > 0.999$. The dynamic ranges of all targets when measured in serum are shown, and there is good correlation of target added to serum and the amount of target measured.

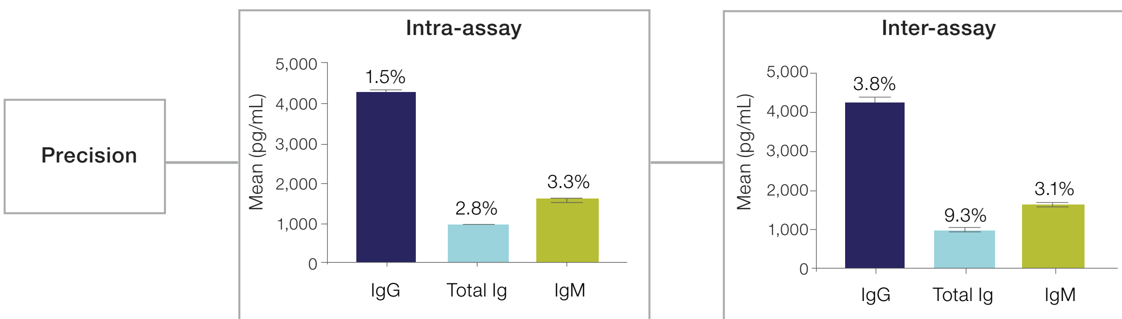


Figure 4. Precision of assay. Intra-assay precision was assessed from data obtained by one operator using one serum batch spiked with different concentrations of IgG, total Ig, and IgM (6 replicates). Inter-assay precision was assessed from data obtained by 3 operators over 3 different days using 3 serum samples that contained different concentrations of IgG, total Ig, and IgM (n = 9 experiments for each serum sample). The coefficient of variation is shown above each bar.

Recovery

The acceptance range for recovery of targets in ELISA is usually 80–120% [1]. The recovery of SARS-CoV-2 IgG, total Ig, and IgM was addressed by spiking three different concentrations of immunoglobulins into serum, covering the dynamic range of each target. The analysis was performed using the ELISA method based on Dynabeads magnetic beads (Table 1). The recovery levels of SARS-CoV-2 IgG, total Ig, and IgM from all three concentrations were largely within the acceptable range of 80–120%. Similar performance was obtained in EDTA plasma, citrate plasma, and heparin plasma (data not shown).

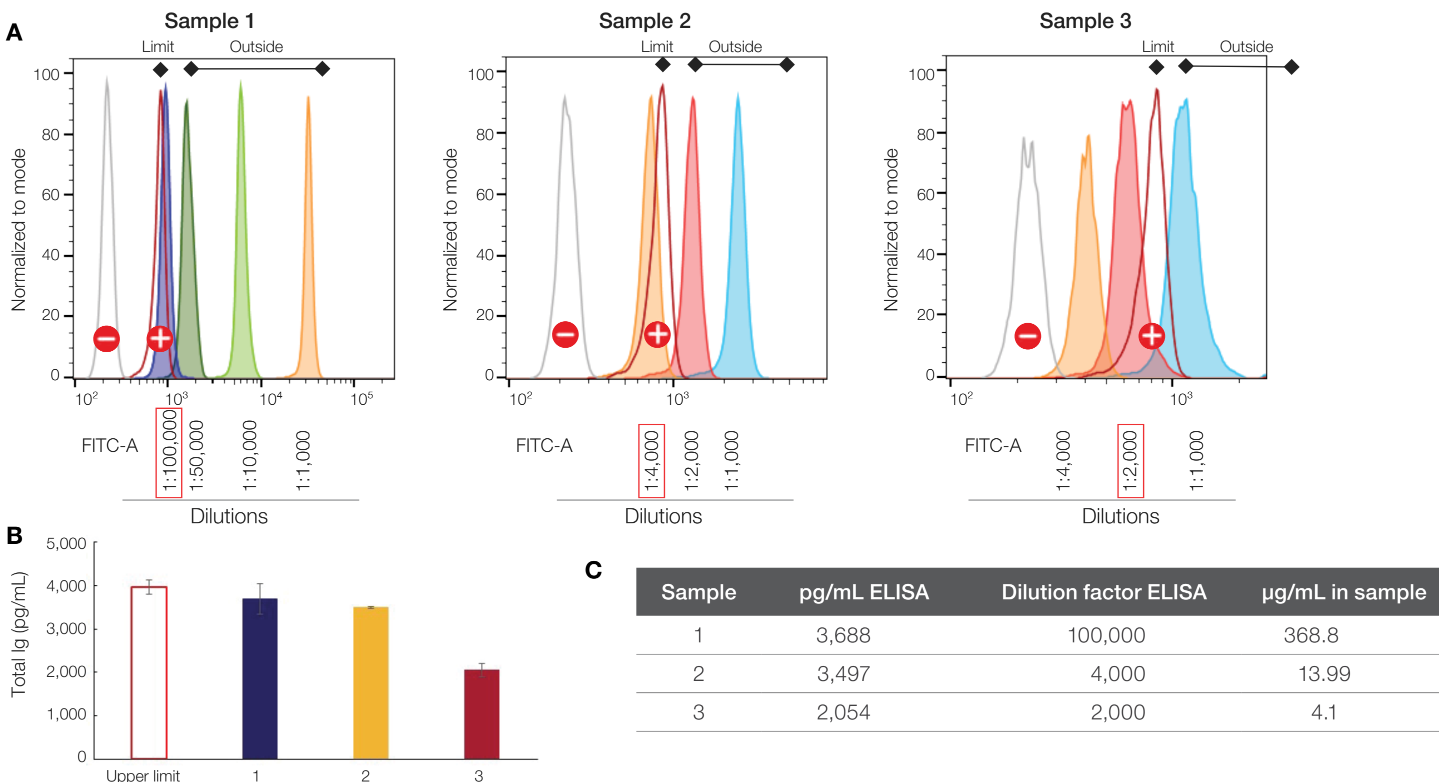
Table 1. Recovery analysis.

Target (in serum)	% recovery (spiked)	% recovery (spiked)	% recovery (spiked)
IgG	102 (15,000 pg/mL)	113 (3,750 pg/mL)	125 (938 pg/mL)
Total Ig	102 (4,000 pg/mL)	96 (1,000 pg/mL)	97 (250 pg/mL)
IgM	103 (30,000 pg/mL)	87 (1,880 pg/mL)	107 (470 pg/mL)

Rapid, quantitative bead-based ELISA for SARS-CoV-2 total Ig in plasma and serum from clinical samples

This rapid bead-based ELISA method was used to quantify the total Ig against SARS-CoV-2 in plasma and serum from three individuals suspected of being exposed to SARS-CoV-2 infection (sample 1: serum; sample 2: plasma; and sample 3: plasma) (Figure 5). We used our newly developed bead-based flow cytometry analysis method to determine the best dilution factor for ELISA. The method can be performed manually or by a hands-off solution using a KingFisher Flex or Apex instrument.

Diluted plasma or serum (100 µL) was incubated with Dynabeads magnetic beads and prepared for flow cytometry. Plasma samples from uninfected individuals were used as negative controls. Beads conjugated with SARS-CoV-2 trimeric spike protein were incubated with negative control samples followed by a detection antibody. As positive controls, SARS-CoV-2 spike protein antibody (1 µg/mL) was spiked into negative control samples at concentrations corresponding to the linear range of the standard curve for the Dynabeads SARS-CoV-2 Spike Ig Total ELISA Kit. The dilutions for all three samples corresponding to the linear range of the ELISA standard curve were used to measure the concentration of total Ig by ELISA (Figure 5B). The dilution factors were used to calculate the concentrations of the SARS-CoV-2 total Ig in the plasma samples (Figure 5C).



Sample	pg/mL ELISA	Dilution factor ELISA	µg/mL in sample
1	3,688	100,000	368.8
2	3,497	4,000	13.99
3	2,054	2,000	4.1

Figure 5. Rapid, flow cytometry–based method for finding the right dilution factor for unknown samples suitable for quantitative ELISA of SARS-CoV-2 total Ig using Dynabeads magnetic beads. (A) The gray peak shows the negative control. The red-line peak is the high control, which is equivalent to the upper limit of the total Ig ELISA standard curve. The other peaks correspond to different dilutions of plasma samples. **(B)** A rapid bead-based ELISA for quantification of total Ig in plasma from the same three unknown samples tested in **A**. The unknown samples were analyzed at the dilutions found in **A** corresponding to the linear range of the total Ig ELISA standard curve. The absorbance was measured at 450 nm, and SkanIt Software was used to calculate the amount of total Ig (pg/mL) in the unknown samples. **(C)** The dilution factor was used to calculate the concentrations of total Ig (pg/mL) in the unknown plasma samples.

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Conclusions

We have described simple, rapid, and reproducible protocols for capture of immunoglobulins against SARS-CoV-2 spike protein from serum or plasma using spike protein–coated Dynabeads magnetic beads. The Dynabeads SARS-CoV-2 Spike magnetic beads are included in the ready-to-go kits for qualitative and quantitative ELISA that can be performed in only 45 minutes, either manually as described here or automated on a KingFisher Flex or Apex instrument. The bead-based ELISA kits have the expected sensitivity, recovery, and dynamic range, but they are performed 2–5 times faster, in a more reproducible manner, and with less hands-on time than standard plate-based ELISAs.

Ordering information

Description	Cat. No.
For flow cytometry assay	
Dynabeads SARS-CoV-2 Spike	18100D
HulaMixer Sample Mixer	15920D
DynaMag-96 Side Skirted Magnet	12027
Attune NxT Flow Cytometer	thermofisher.com/attune
For ELISA	
Dynabeads SARS-CoV-2 Spike Ig Total ELISA Kit	18020D
Dynabeads SARS-CoV-2 Spike IgM ELISA Kit	18010D
Dynabeads SARS-CoV-2 Spike IgG ELISA Kit	18000D
White U-bottom microplate, Greiner	650207 (fishersci.com)
DynaMag-96 Side Skirted Magnet	12027
Clear Flat-Bottom Immuno Nonsterile 96-Well Plates	439454
Varioskan LUX Multimode Microplate Reader	VLBLATD2
For automation	
KingFisher Flex Purification System with 96 Deep-Well Head	5400630
KingFisher Plastics, for 96 deep-well format, V-bottom	95040450
KingFisher 96 Tip Comb for Deep-Well Magnets	97002534
KingFisher Apex Purification System with 96 Deep-Well Head	5400930

Reference

1. Andreasson U et al. (2015) A practical guide to immunoassay method validation. *Front Neurol* 6:179.

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