

Efficient and gentle processing of magnetic Dynabeads®

using Tecan's HydroFlex™ microplate washer



Introduction

The use of magnetic beads as the solid phase in ELISA (Enzyme-linked immunosorbent assay) has several advantages over direct coupling to the wells. These advantages include an increased available surface area and an even distribution of beads throughout the sample providing rapid and sensitive detection of low analyte concentrations (1. Liabakk et al).

When Dynabeads® are used as the solid phase, the capture antibody is coupled to the beads in bulk, which ensures high reproducibility and eliminates the need to perform QC of each plate with antibody coupling.

Magnetic beads have thus become the gold standard for companies that provide and develop immunodiagnostic assays. By automating tedious manual wash steps, bead-based ELISAs may also get used in research labs in the future.

Using Dynabeads® in combination with the HydroFlex™ plate washer configured for magnetic bead washing, it is possible to run bead-based ELISA with the convenience of the 96-well plate format and the ease of handling known from traditional well-based ELISA.

In this application note we describe the use of Tecan's HydroFlex™ washer equipped with the smart-2 MBS magnetic carrier for automated washing of Dynabeads® in an ELISA.

The model system used for this application note was a simple sandwich assay (Figure 1) designed to compare the automated processing of magnetic beads using the HydroFlex™ with the traditional manual procedure.

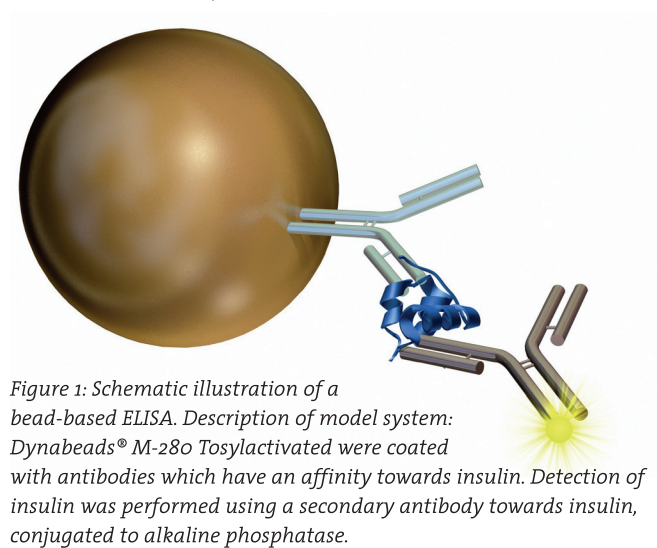


Figure 1: Schematic illustration of a bead-based ELISA. Description of model system: Dynabeads® M-280 Tosylactivated were coated with antibodies which have an affinity towards insulin. Detection of insulin was performed using a secondary antibody towards insulin, conjugated to alkaline phosphatase.

Material and Methods

Instruments

- Tecan HydroFlex™ plate washer configured with an 8-channel wash head, 4 inlet-channels for wash buffers and the optional smart-2 MBS carrier for washing of magnetic beads
- Microplate reader for detection of chemiluminescence

Dynabeads® and Microplates

- Dynabeads® M-280 Tosylactivated (Invitrogen Dynal AS, Norway)
- Greiner 96-well microplate, flat bottom, white (Greiner Bio-One, Germany, article code 655207)
- Corning® 96-well microplate, round bottom, white (Corning, US, article code 3355)

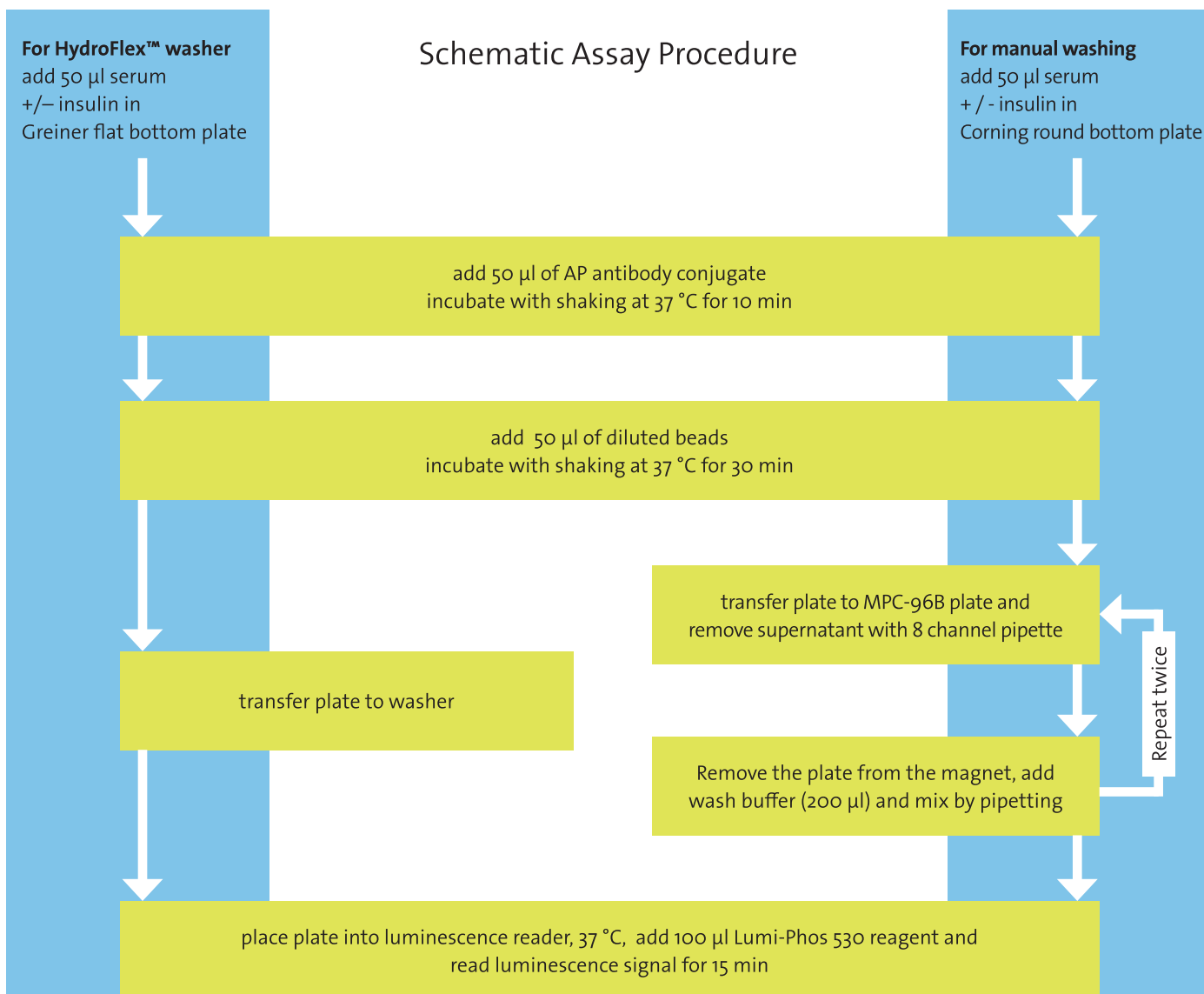


Figure 2: Schematic illustration of the experimental setup comparing the automated washing on the Tecan HydroFlex™ plate washer (left) and the manual wash procedure (right).

Due to the automation of the entire wash procedure the number of hands-on steps with the HydroFlex™ plate washer is substantially smaller compared to the manual wash procedure with a pipette.

Assay Procedure in detail

Human Insulin sandwich immunoassay:

- a) Anti-insulin mAb 1 (clone 7F8, Hytest) was bound to Dynabeads® M-280 Tosylactivated in two different concentrations (5 and 25 µg/mg beads) according to manufacturers instructions
- b) Anti-insulin mAb 2 (clone D4B8, Hytest) was labeled with alkaline phosphatase using AbD Serotec LYNX Rapid Alkaline Phosphatase Antibody Conjugation Kit® (LNK012AP)
- c) Recombinant human insulin (Sigma I2643) was dissolved in human serum (Sigma S7023) to 50 ng/ml
- d) Beads with bound antibody (step a) were diluted to 0.4 mg/ml in "DELFI[®] Assay Buffer" (PerkinElmer Inc., #1244-111) and incubated at roller for 30 minutes
- e) I) For HydroFlex™: 50 µl serum with and without added insulin (step c) was placed in replicates of 4 in a white, flat bottom 96 well plate (Greiner # 655207)
II) For manual: 50 µl serum with and without added insulin (step c) was placed in replicates of 4 in a white, round bottom 96-well plate (Corning # 3355)
- f) 50 µl of alkaline phosphatase-antibody conjugate (step b) diluted 1:1000 (approx 5 ng/ml) in "DELFI[®] Assay Buffer" was added to the wells and the plate incubated with shaking at 37°C for 10 minutes
- g) 50 µl (20 µg) of the diluted beads (step d) were added to the wells and the plate incubated with shaking at 37°C for 30 minutes
- h) I) For HydroFlex™: Automated washing of the plate on the HydroFlex™ using the program "Inv6_mag" and "DELFI[®] Wash Buffer" (PerkinElmer Inc., #1244-114) in channel 1.
II) For manual: The plate was washed manually using a 8 channel pipette and a MPC™-96B magnet (Invitrogen Dynal AS, Norway). Three washes with "DELFI[®] Wash Buffer" with resuspension of the beads at each dispensing step were performed. The plate was then left on the magnet for 30 seconds before aspirating the supernatant
- i) The plate was transferred to a chemiluminescence reader pre-heated to 37 °C. 100 µl Lumi-Phos® 530 (Lumigen, Inc.) was added to the wells and the increase in luminescent signal monitored for 15 minutes
- j) The slope of the curve (RLU/min) was calculated and used for comparing the samples

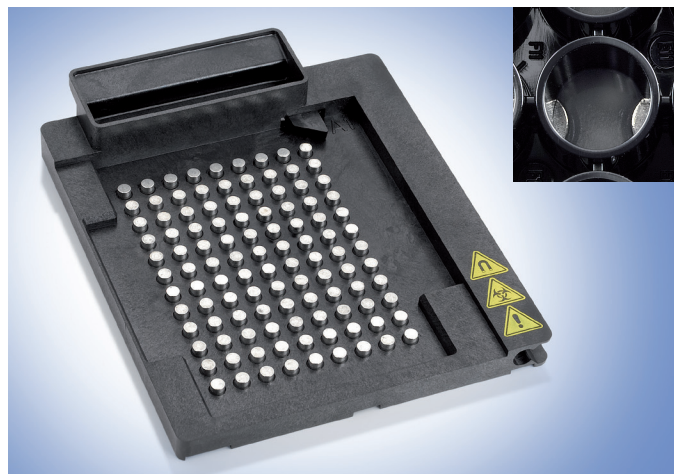


Figure 3: Optional smart-2 MBS carrier for automated washing of Dynabeads® with Tecan's HydroFlex™.

Instrument Settings

Wash Program "Inv6_mag"	Parameters
CYCLE 1	# of cycles: 1
- Soak	60 s, shaking @ medium intens.
CYCLE 2	# of cycles: 2
- Aspirate: z-pos. custom: 4 mm	1 s, asp. rate:1, head sp. 8 mm/s
- Dispense: z-pos. overflow	Ch.1, 200 µl, disp. rate: 300 µl/s
- Soak	60 s, shaking @ medium intens.
CYCLE 3	# of cycles: 1
- Aspirate: z-pos. custom: 4 mm	1 s, asp. rate:1, head sp. 8 mm/s
- Dispense: z-pos. overflow	Ch.2, 200 µl, disp. rate: 300 µl/s
- Soak	60 s, shaking @ medium intens.
- Final Asp.: crosswise, z-pos. custom 6.3 mm	1 s, asp. rate:1, head sp. 8 mm/s

Table 1: Wash program tested for magnetic bead purification using the HydroFlex™ microplate washer.

Results

The chemiluminescent signals of a Dynabeads® based human Insulin sandwich Immunoassay were compared using an automated wash procedure provided by the Tecan HydroFlex™ microplate washer and a manual wash procedure via a multichannel pipette.

Additionally, the effect of two different concentrations of antibodies coupled to the Dynabeads® were compared. (see Figure 4 and Figure 5)

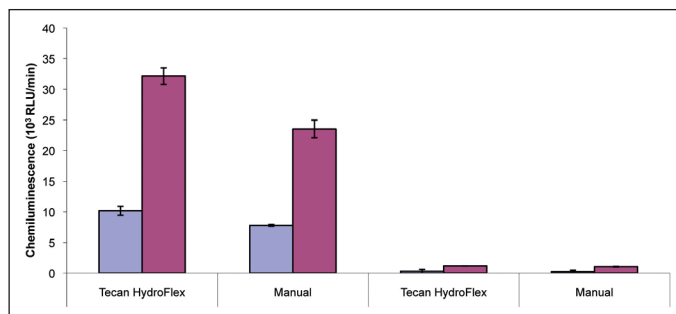


Figure 4: Chemiluminescent signals from two different concentrations (■ 5 and ■ 25 µg Ab/mg beads) of antibodies coupled to Dynabeads® processed automatically using Tecan's HydroFlex™ washer and processed manually. Both positive and negative controls give specific signals, due to the presence of insulin in the negative control serum.

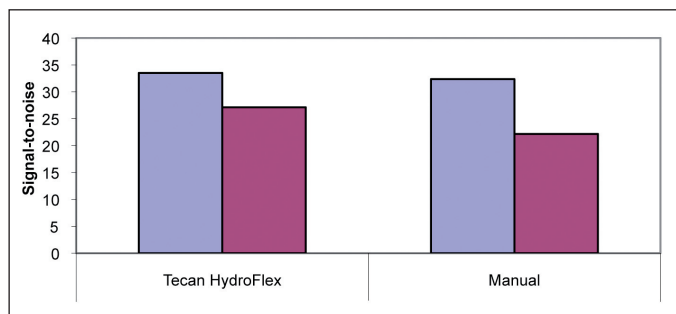


Figure 5: Signal-to-noise ratio for the two different Ab coupled Dynabeads® (■ 5 and ■ 25 µg Ab/mg beads) comparing the automated and manual protocol. The signal-to-noise ratio is better for the automated washing procedure using Tecan's HydroFlex™ compared to the manual washing.

Conclusion

The HydroFlex™ microplate washer was successfully used to automate time consuming wash steps of an ELISA-assay based on magnetic Dynabeads®, which up to now had to be performed manually using a multichannel pipette.

Dynabeads® in combination with the HydroFlex™ plate washer make it possible to run magnetic bead based ELISA with the convenience offered by the 96-well plate format and the ease of handling known from traditional well based ELISA.

The results showed no significant differences in the standard deviations or signal-to-noise ratios, indicating that the wash efficiency for the automated procedure with the HydroFlex™ plate washer is as good as with the manual procedure, where the Dynabeads® beads must be fully resuspended in each wash cycle.

The signals obtained using the HydroFlex™ plate washer were slightly higher than for the manual wash procedure using a multi-channel pipette, indicating a slightly lower loss of beads (or detection antibody) during the automated wash process. This shows that the automated wash is gentle and effective.

Low positive values were expected for the negative samples since some insulin was present in the human serum used in this study.

Acknowledgements

We express our acknowledgements to Mr. Erling Finne and Mrs. Ingrid Manger for the assay optimization, analysis of the data and writing this note.

For more information please see:

- www.invitrogen.com/dynabeads
- www.tecan.com/HydroFlex

Literature

1. Nina-Beate Liabakk, Kjell Nustad, Terje Espevik (1990) A rapid and sensitive immunoassay for tumor necrosis factor using magnetic monodisperse polymer particles. Journal of Immunological Methods, 134, 2, 253-259

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