Silica-Like Dynabeads® – A Versatile Platform for Viral Nucleic Acid Capture

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Introduction

Novel virus discovery and viral diagnostics have geared more towards molecular assays. In the face of new viral threats and emerging pandemics, methods enabling efficient screening of large populations are vital for speedy discovery and containment of these threats. For this, automated platforms with predominantly magnetic solid phases are used for isolating viral nucleic acids (vNA) from patient samples for downstream molecular assays. The successful development of an automated protocol requires a solid phase which is both functional and reproducible. With this in mind, we have developed Dynabeads® MyOne™ SILANE.

Figure 1. Dynabeads® MyOne™ SILANE morphology

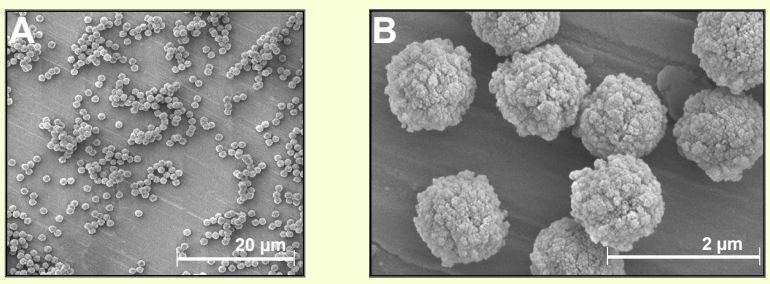

Figure 1. Scanning electron microscopy images of Dynabeads® SILANE show that the beads are monodisperse with 1µm diameter **(A).** Thanks to the small uniform size and the ruffled surface with silica-like chemistry **(B)**, the beads have large and well-defined surface area (20m²/g) that offers excellent reaction kinetics when binding even only a few copies of vNA.

Figure 2. Dynabeads® MyOne™ SILANE size distribution and sedimentation rate

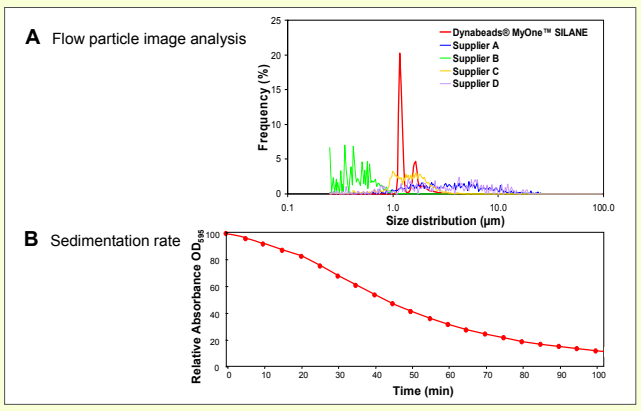

Figure 2. Flow particle image analysis (Sysmex FPIA 3000) shows two distinct peaks for Dynabeads® SILANE **(A; red line)**: The major peak represents monodisperse beads and the smaller peak shows dimers. In comparison, magnetic particles from other suppliers show great variations in size **(A; blue, green, yellow, and purple lines)**.

The sedimentation rate of Dynabeads® SILANE was measured by setting the OD_{595} of fully suspended beads at 100%, then the changes in absorbance over time were given relative to the fully suspended beads. Here, Dynabeads® SILANE show very little change in absorbance within the first 10 minutes (pipetting time)**(B)**. Dynabeads® SILANE also have increased iron content compared to the other beads in the Dynal® portfolio, which improves the magnetic recovery in viscous samples (data not shown). Together, the above features make Dynabeads® SILANE very suitable for automated platforms.

Figure 3. Method: vNA isolation using Dynabeads® MyOne™ SILANE

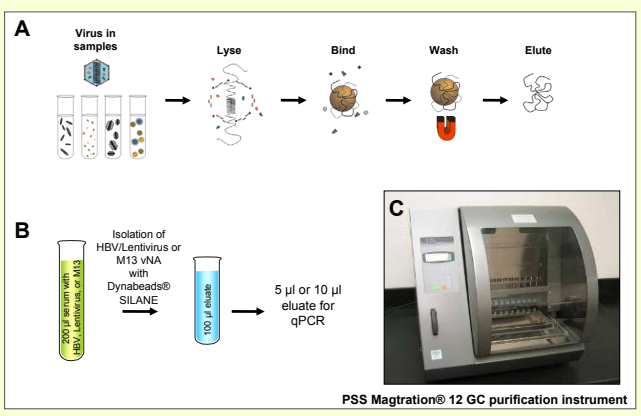

Figure 3. Protocols were developed for both manual isolation and the automated platform, the Precision Science System (PSS) Magtration® 12 GC purification instrument **(A and C)**. Briefly, 200 µl human and bovine sera were laced with dilutions of Lentivirus or serum from Hepatitis B virus (HBV) infected patients, and M13 bacteriophages, respectively **(B)**. The vNA was isolated either manually (M13) or by the PSS instrument (HBV and Lentivirus) and evaluated by quantitative PCR (qPCR).

Summary

Dynabeads® MyOne™ SILANE:

- Are small (1µm) monodisperse magnetic beads with uniform size and well-defined surface area with low sedimentation rate.
- Show high batch to batch reproducibility for vNA isolations of both RNA and DNA viruses.
- Isolate vNA over a very broad titer range with similar high recovery rate over the entire range.
- Work efficiently and reproducibly in the PSS Magtration® 12 GC instrument.

Figure 4. M13 bacteriophage serial dilution isolation quantification by Real-Time PCR

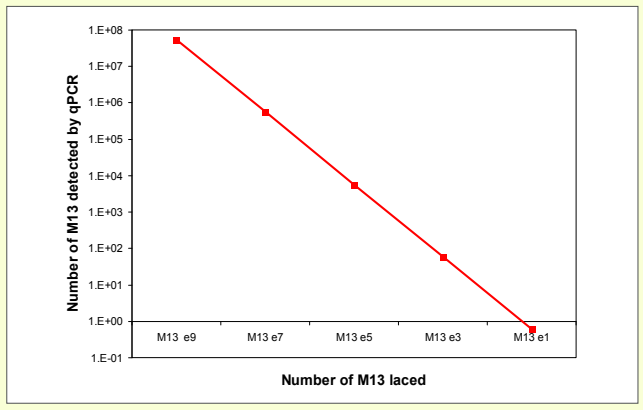

Figure 4. M13 bacteriophages were serially diluted, where 1.37 x 10^9 pfu, 1.37 x 10^7 pfu, 1.37 x 10^5 pfu, 1.37 x 10^3 pfu, and 13.7 pfu were used to lace 200 µl bovine serum for manual isolation. Five µl of the recovered vNA from the total elution volume of 100 µl was analyzed by qPCR (AB7500 Real-Time PCR system, Applied Biosystems). The same M13 dilution series used to lace the serum was also used as the qPCR standard curve. (Note that the vNA isolation elution introduces a 10 fold dilution.) As the graph shows, viral detection was possible in all dilutions, over 9 exponential points, with the lowest detection limit being mere a few copies of vNA.

Viral NA was also isolated by PSS Magtration® 12 GC instrument from human serum laced with serially diluted Lentivirus and HBV infected patient serum (both dilution series ranging from 10^3 to 10^0 virions). Similar to the M13 results, detection of both Lentivirus and HBV were possible in all dilutions tested (data not shown). Our results indicate that Dynabeads® SILANE can isolate the vNA from both RNA and DNA viruses with high sensitivity over a very broad titer range.

Figure 5. M13 bacteriophage serial dilution recovery rate

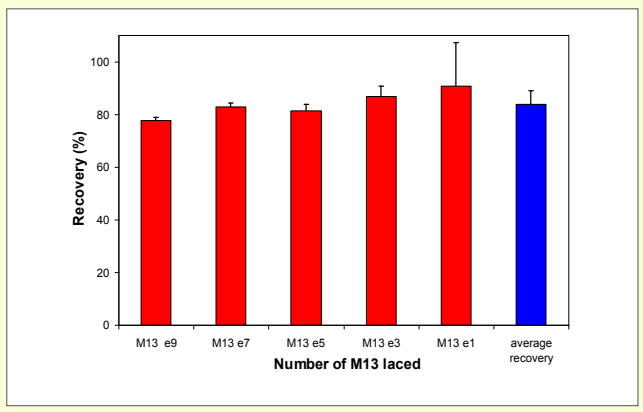

Figure 5. M13 recovery rates of a manual isolation were evaluated. The bar graph shows the recovery rates over 9 exponential points. The recovered vNA is given as percentage of the amount of M13 used to lace the serum. As can be seen, these results show that the recovery rate is similar for all dilutions, with the average recovery rate being 84 %. This indicates that Dynabeads® SILANE's recovery rate is most likely uniformly efficient over a very broad titer range. (Note that this vNA extraction was done manually, which may introduce more tube to tube variation than that of an automated platform.)

Figure 6. Dynabeads® MyOne™ SILANE vNA recovery batch to batch variation

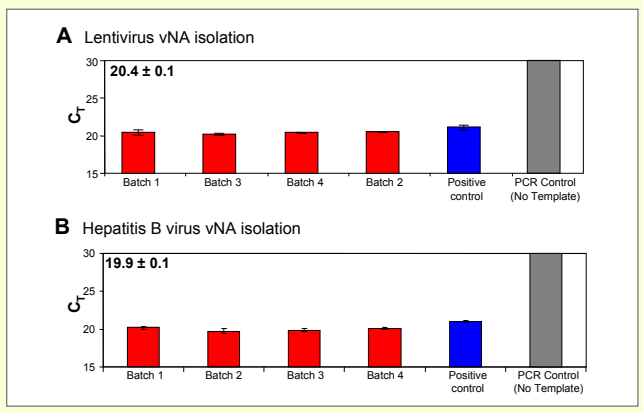

Figure 6. Four large scale batches of Dynabeads® SILANE were tested for batch to batch variability in vNA isolation. Viral NA was isolated from human serum laced with Lentivirus (~1 x 10^{10} virions) and HBV (~6 x 10^6 virions) by PSS Magtration® 12 GC instrument. A commercially available manual spin-column was used in parallel as positive control. The recovered vNA was analyzed by qPCR. The graphs show the CT values of the four Dynabeads® SILANE batches (red bars) in comparison to the manual spin-column (blue bars) for the Lentivirus isolation **(A)** and HBV isolation **(B)**. As can be seen, the variation between batches is negligible for both RNA and DNA virus isolations, showing high reproducibility. Viral NA isolated with Dynabeads® SILANE also shows lower CT values than that of the spin-column, indicating more efficient recovery.

Conclusion

The above features make Dynabeads® MyOne™ SILANE a highly functional magnetic solid phase, well-suited for vNA isolation in automated platforms. These features will enable reliable high-throughput molecular assays for mass-screening large population samples.