Sensitive and automation-friendly isolation of viral RNA/DNA using Dynabeads® SILANE

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Introduction

Technologies for nucleic acid isolation have evolved. The use of automated systems allows for more robust, systematic, and efficient capture of nucleic acids, with reduced labor time and reagent needs.

Invitrogen has developed a new Dynal® product for automation-friendly isolation of viral nucleic acids (NA). The Dynabeads® SILANE viral NA kit offers enhanced performance beyond the capabilities offered by alternative magnetic separation systems. The procedure is rapid, reliable, and well suited for automated high-sensitivity assays. This product has been developed to meet the rigorous requirements of solid-phase sample preparation for diagnostic and biotech OEM customers.

Dynabeads® magnetic separation technology

By pioneering biomagnetic separation technology in the 1980s, Dynal® revolutionized separation methodologies. Magnetic particles from other suppliers often have random size distributions, surface areas, and binding capacities (Figures 1A and 2). This variability could compromise the reproducibility of your assay results.

Dynabeads® are manufactured under highly controlled and validated production processes. The resulting magnetic beads have a uniform size and defined silica-like surface (Figures 1B and 2). This will ensure optimal performance with high sensitivity and reproducibility in your automated assays.

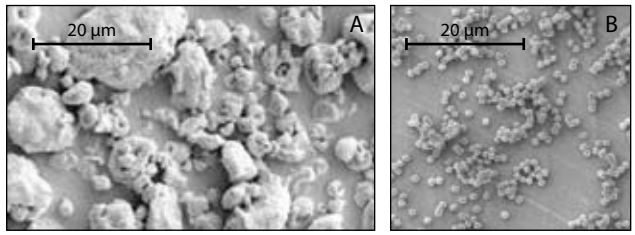

Figure 1—No compromises on reproducibility. The random sizes and surface areas of magnetic particles from other suppliers **(A)** could compromise the reproducibility of your assay results. Dynabeads® MyOne™ SILANE **(B)** are monosized, 1 µm magnetic beads with a large, well-defined surface area, providing highly reproducible results.

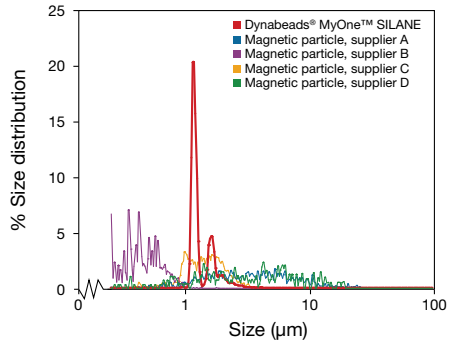

Figure 2—Dynabeads® are monosized. The graph shows the size frequency distribution (in percent) of Dynabeads® MyOne™ SILANE and magnetic particles from four alternative suppliers, as analyzed by flow particle image analysis on a Sysmex FPIA-3000 (Malvern Instruments, Ltd.). The second peak for Dynabeads® at 2 µm represents doublets. Monosized Dynabeads® ensure optimal behavior in automated systems. Minimal variation within and between lots allows for standardized and reproducible processes.

Materials and methods

Product description

The Dynabeads® SILANE viral NA kit (Cat. no. 370-11D) contains Dynabeads® MyOne™ SILANE (5 ml, 40 mg/ml); lysis/binding, washing, and elution buffers; and a protocol optimized for efficient isolation of low-titer viral DNA or RNA from biological samples (e.g., serum and plasma). The kit contains reagents sufficient for up to 96 isolations.

Dynabeads® MyOne™ SILANE are uniform, monosized magnetic beads, 1 µm in diameter. They are composed of highly crosslinked polystyrene with evenly distributed magnetic material. The beads have an increased magnetic strength compared to the main Dynabeads® portfolio, which ensures rapid magnetic mobility. Their defined surface area and optimized silica-like surface chemistry offer excellent reaction kinetics when binding even only a few copies of viral DNA/RNA. Together with their small size, these features allow for efficient isolation of viral DNA or RNA in viscous samples such as serum. The beads also feature a low sedimentation rate and favorable reaction kinetics, making them particularly well suited for automated liquid handling.

Protocol description

The Dynabeads® SILANE viral NA kit is an excellent tool for isolation of viral DNA or RNA from low viral titer samples, following a simple and scalable protocol (Figure 3). A lysis buffer is first added to the sample, followed by incubation with Dynabeads® MyOne™ SILANE (typically 2 mg beads per 200 µl sample volume). When placed in a magnetic field, the beads with bound DNA or RNA are collected at the tube wall and unbound material is removed by aspiration. Magnetic separation facilitates simple buffer changes and elution of the isolated DNA or RNA. Dynabeads® protocols are flexible and easily adapted to automated liquid handling platforms.

Dynabeads® MyOne™ SILANE are chemically stable and can be used with a wide variety of buffer systems. The best isolation results are achieved with the buffers supplied in the kit (Figure 4). Another set of buffers has been developed for isolation of genomic DNA and is part of the Dynabeads® SILANE genomic DNA kit (Cat. no. 370-12D). Please contact Invitrogen if you would like to discuss specific applications and system development, validation, or a potential OEM arrangement.

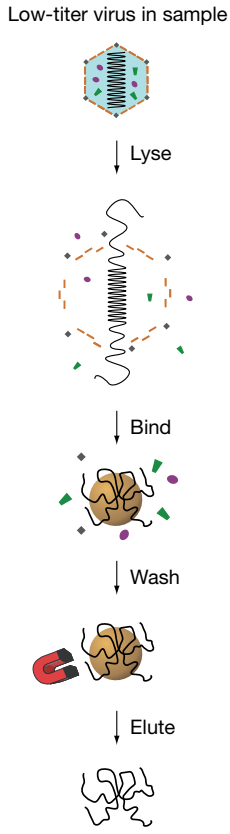

Figure 3—Quick and automatable magnetic separation protocol. The illustration shows the simple steps (lyse, bind, wash, and elute) of the protocol for isolating viral DNA or RNA from biological samples using the Dynabeads® SILANE viral NA kit.

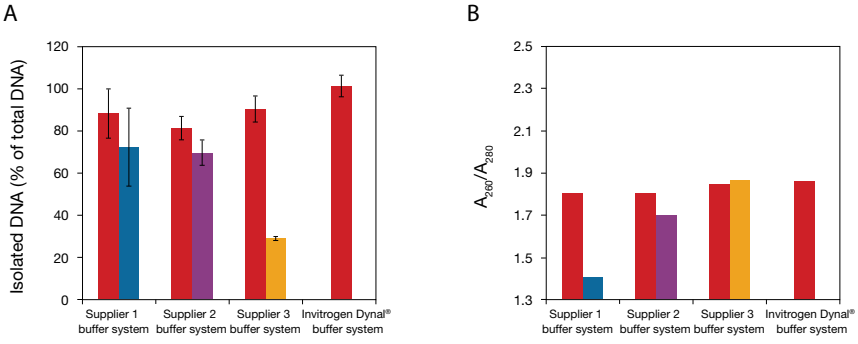

Figure 4—Dynabeads® can be used with different buffer systems. The graphs show **(A)** specific yield and **(B)** purity of DNA isolated using alternative magnetic particles and buffer systems. Human serum (100 µl) spiked with a lambda phage DNA ladder (5 µg) was used as a model system. Comparable results were seen with an RNA ladder (not shown). Dynabeads® MyOne™ SILANE (red) outperform the alternative magnetic particles (blue, purple, and orange). Note that Dynabeads® MyOne™ SILANE give better yield and purity than the alternative separation products, even in their respective buffer systems.

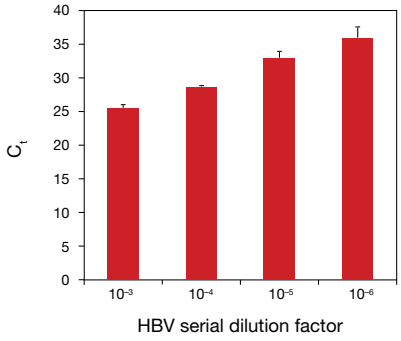

Figure 5—Linear scalability of HVB isolation procedure. HBV DNA was isolated from patient sera infected with HBV virus, using the Dynabeads® SILANE viral NA kit developed as an automated protocol on a PSS platform (Precision System Science). HBV-infected serum was serially diluted: 10^{-3} (1:1,000), 10^{-4}, 10^{-5}, and 10^{-6}. The isolated HBV DNA was detected by quantitative PCR. The observed data closely match the theoretical expected data, with a C_t difference between 10-fold dilutions of ~3.3.*

Results

The results shown demonstrate how Dynabeads® MyOne™ SILANE improve viral nucleic acid isolation.

Dynabeads® MyOne™ SILANE demonstrate clear linear scalability, where the amount of viral RNA or DNA isolated is directly proportional to the amount present in the sample (Figure 5). The isolation procedure performs well with DNA viruses, including challenging viruses such as HBV (Figures 5 and 6), and RNA viruses (Figure 8).

When isolating viral DNA from low-titer samples, Dynabeads® MyOne™ SILANE outperform the other magnetic separation products (silica particles) tested. HBV DNA detection was feasible in samples containing 40 infectious units only when Dynabeads® were used (Figure 6). Fewer Dynabeads® are required to obtain similar results (typically 15–20% less), compared to magnetic particles from an alternative supplier.

The lower detection limit of Dynabeads® MyOne™ SILANE for a 220 bp HBV DNA construct was also shown to be superior to that of a silica spin-column system (Figure 7).

The size (Figures 1B and 2) and iron content of the monodisperse Dynabeads® are tightly controlled. This secures a high level of reproducibility, reliable pipetting, and optimal performance. The isolation procedure can be adopted to both open and closed automated systems (Figure 8).

The data presented here are from model systems testing bead and buffer functionality, and do not reflect the results of an optimized automated molecular assay. Please contact us to discuss a potential OEM arrangement for protocol automation and product customization.

Summary

Sensitive and highly reproducible

Dynabeads® MyOne™ SILANE offer reputable Dynal® high standards with unique intra- and inter-lot reproducibility. This ensures:

- Superior batch-to-batch reproducibility that reduces assay variability
- Higher reproducibility than with magnetic particles from alternative suppliers
- Higher sensitivity (fewer false negatives), allowing for a low detection limit in real-time PCR assays
- Less Dynabeads® are required to obtain similar results (typically 15–20% less)
- The procedure works well with both DNA and RNA viruses
- Flexible sample, reaction, and elution volumes
- A quick protocol with reduced manual handling
- Automation-friendly protocols (slow sedimentation rate and high magnetic mobility)
- Integration of sample preparation with open and closed automated systems

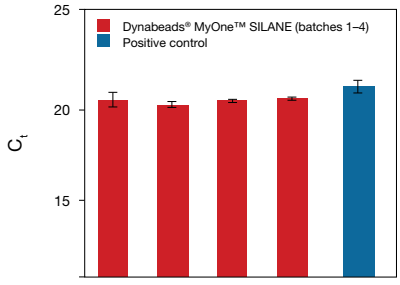

Figure 8—Sensitive and reproducible isolation of lentivirus RNA. The Dynabeads® SILANE Viral NA kit (4 different batches, red columns) was used to isolate lentivirus RNA from spiked human plasma samples. The extractions were done on the iPrep™ automated platform (Invitrogen). The positive control (blue column) was a manual extraction using the PureLink™ Viral RNA/DNA Mini Kit (Invitrogen). High sensitivity (low C_t values) and outstanding reproducibility are seen across the different Dynabeads® MyOne™ SILANE batches.

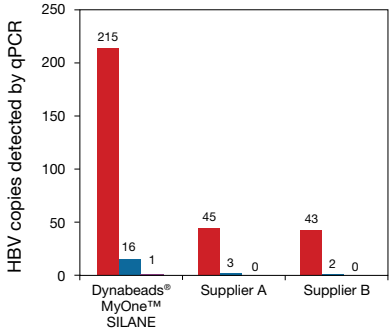

Figure 6—Sensitive detection of HBV virus. Known amounts of HBV—4,000 (red), 400 (blue), and 40 (purple) copies—were spiked into 200 µl serum samples. The isolated HBV DNA was eluted in 100 µl, of which 10 µl (equivalent to 400, 40, and 4 copies, assuming 100% recovery) was used for qPCR. Only Dynabeads® MyOne™ SILANE enabled HBV detection in isolations with 40 copies per 200 µl serum (4 copies in PCR).*

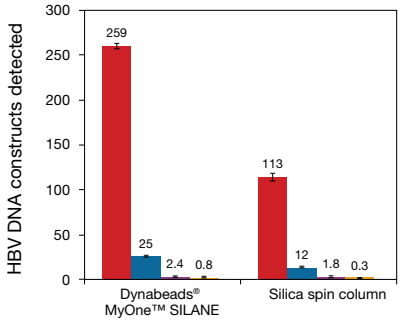

Figure 7—Sensitive detection of a 220 bp HBV DNA construct. Known amounts of an HBV DNA construct—12,000 (red), 1,200 (blue), 120 (purple), and 12 (orange) copies—were spiked into 100 µl serum samples. The isolated HBV DNA was eluted in 100 µl, of which 5 µl (equivalent to 600, 60, 6, and 0.6 copies, assuming 100% recovery), was used for qPCR. Dynabeads® MyOne™ SILANE show a higher sensitivity compared to spin columns.

* We would like to acknowledge Dr. Einar Sverre Berg at the Norwegian Institute of Public Health, Oslo, Norway, for the work shown in Figures 5 and 6.

Ordering information

Product	Quantity*	Cat. no.
Dynabeads® SILANE viral NA Contains Dynabeads® MyOne™ SILANE and specific buffers optimized for sensitive isolation of viral DNA/RNA from human serum/plasma samples	96 isolations	370-11D
Related products		
Dynabeads® MyOne™ SILANE Manufactured under validated production processes; can be supplied in bulk quantities	5 ml (40 mg/ml)	370-02D
Dynabeads® SILANE genomic DNA Contains Dynabeads® MyOne™ SILANE and specific buffers optimized for predictable isolation of pure genomic DNA from human whole blood	96 isolations	370-12D

* Alternative product formats are available.

Dynabeads® for specific capture of nucleic acids

A comprehensive range of Dynabeads® for specific capture of nucleic acids is also available, with different bead sizes and surface functionalities. Some Dynabeads® are precoated with streptavidin, allowing for capture of biotinylated nucleic acids in a wide variety of protocols. Other Dynabeads® have a specific surface chemistry for coupling nucleic acids (e.g., hybridization probes and primers) and/or other ligands.

Custom development

Our strong assay development and immobilization competencies enable us to respond to our customers' needs and work with you for system development, validation, and customization on an OEM level.

If you would like to discuss a potential collaboration or OEM agreement, please contact us at ivd@invitrogen.com.