Rapid bead-based viral enrichment for multiomic viral research

Optimizing with Dynabeads Intact Virus Enrichment

Keywords

Dynabeads, enrichment, SARS-CoV-2, virus-like particles, western blot, qPCR, plaque assay, viral transport medium, cell culture medium, wastewater, influenza virus A (INFV), H1N1, respiratory syncytial virus, Zika virus, adenovirus, enterovirus, norovirus

In this application note, we show:

- A solution for the rapid enrichment of intact viruses for multiomic viral research
- How isolation by magnetic bead–based enrichment, including from very dilute samples, can provide greater yields of intact viruses essential for viral research
- A simple, fast, and reliable method for the isolation of intact SARS-CoV-2 virus, influenza virus A, respiratory syncytial virus, Zika virus, adenovirus, enterovirus, and norovirus particles for manual or automated handling

Introduction

Virus enrichment is an essential method for obtaining viruses in the quantities often required to study their life cycles and pathogenesis. Multiomic study of viruses requires isolation of intact virus particles, often from large volumes and very dilute samples. To obtain sufficient amounts of viruses, stocks of virus can be made by inoculation of cell cultures with a seed virus. The infected cells will release new viral particles into the cell culture medium (CCM) at the end of the viral life cycle. The released viral particles are then extracted from the medium. Enrichment of viruses is challenging because of their size and typical concentrations in samples. Virus enrichment can be a tedious and difficult process, and may result in insufficient yield. Low virus titers can introduce artificial variants, or bias in gene sequences. A range of methods that include different forms of ultracentrifugation, precipitation, and filtration are used to enrich virus particles from cell culture supernatants; these methods require expensive instruments, are very time-consuming, and may result in low yields.

Fast and simple virus enrichment

Here we describe a manual workflow that takes less than 15 minutes to enrich intact viruses from dilute cell culture, viral transport medium (VTM), and wastewater, with an optional step (~10 minutes) to release the virus from beads. This short and simple enrichment approach helps reduce the risk of lower yields and affecting the integrity and infectivity of the virus. One of the key features of Invitrogen™ Dynabeads™ magnetic beads in any enrichment or isolation protocol is the rapid binding kinetics. The proximity of the beads to the targets in the solution translates directly to short incubation times and faster protocols (Figure 1A). Here we utilize the physical properties of viruses (negatively charged) in combination with positively charged Invitrogen™ Dynabeads™ Intact Virus Enrichment beads, which leads to charge-based binding without the need for antibody labeling (Figure 1B). This approach can also capture other negatively charged vesicles (e.g., exosomes) or proteins, within 10 minutes (Figure 2A). Following capture, the viruses can be released from the beads by adding an anion with a stronger relative affinity than that of the virus.

This short and easy enrichment approach can be simplified further by using the Thermo Scientific™ KingFisher™ Purification System (Figure 2B). The enriched viruses can be used for functional studies, immunological studies, protein analysis (e.g., western blot), or nucleic acid (NA) extraction (e.g., for qRT-PCR). For details on the manual and automated protocols, see Dynabeads Intact Virus Enrichment.

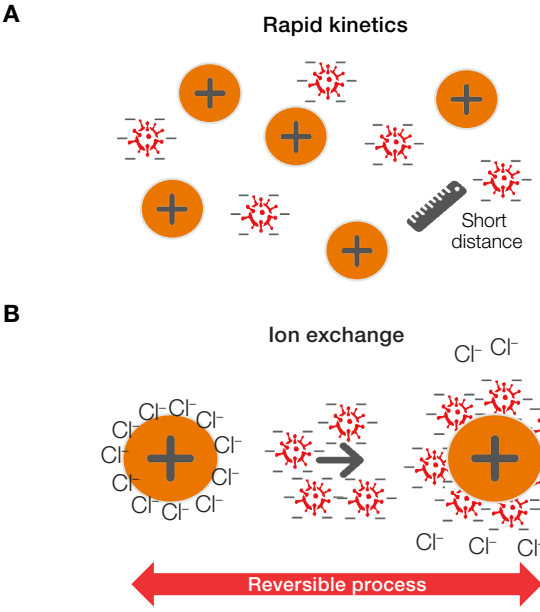

Figure 1. Binding kinetics and principle of enrichment. (A) The positively charged Dynabeads Intact Virus Enrichment beads are close to the negatively charged virus particles, resulting in rapid binding kinetics and a fast enrichment protocol. Dynabeads Intact Virus Enrichment beads are protected with Cl^- ions. Virus particles added to the magnetic beads will displace the Cl^- ions and bind to the bead surface. **(B)** For virus release, an anion with higher relative affinity can be added to displace the viruses and thus release them into the sample. For release, 20 mM triethanolamine with 0.25 M KI or 50 mM citric acid in 50 mM Na phosphate, pH 4, can be used.

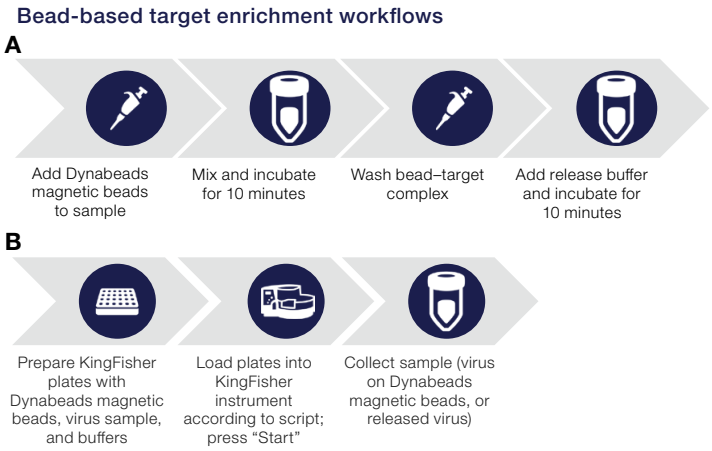

Figure 2. Overview of the bead-based target enrichment workflows. (A) Manual and **(B)** automated workflows.

Outline of viral enrichment and analysis

This study demonstrates the use of the positively charged Dynabeads Intact Virus Enrichment beads to efficiently enrich SARS-CoV-2 from various starting matrices, such as viral transport medium, cell culture medium, or wastewater spiked with virus-like particles or inactivated viruses. The enrichment facilitates downstream analysis by techniques like qRT-PCR and western blot. Additionally, the infectivity of the enriched virus was assessed by plaque assays. This demonstrates the potential of positively charged beads in facilitating viral enrichment and subsequent analysis in various research applications.

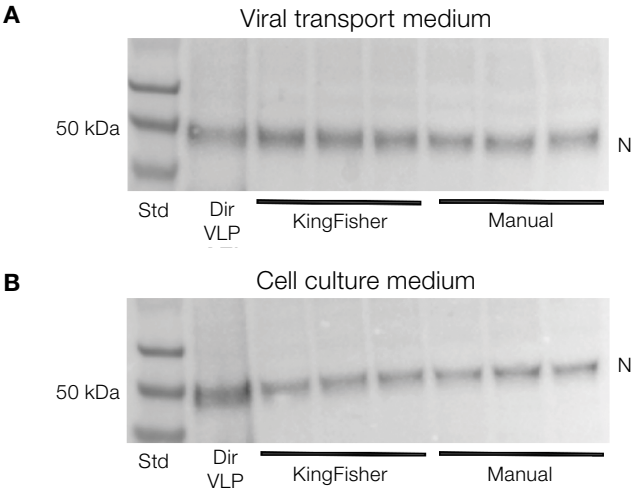

Figure 3. Enrichment of SARS-CoV-2 virus-like particles (VLPs) using Dynabeads Intact Virus Enrichment beads. VLPs were spiked into **(A)** viral transport medium and **(B)** cell culture medium and were captured with Dynabeads Intact Virus Enrichment beads using manual and automated protocols. In both, SARS-CoV-2 nucleocapsid protein (N) was detected by western blot.

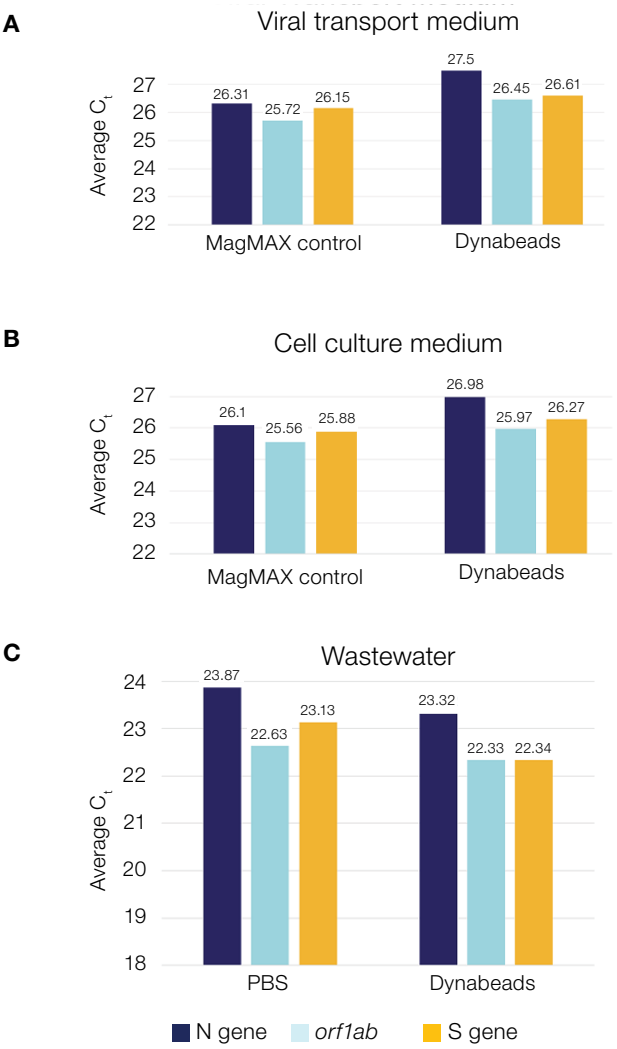

Figure 4. Enrichment of heat-inactivated SARS-CoV-2 virus. The virus was enriched from **(A)** VTM, **(B)** CCM, and **(C)** wastewater. The N, *orf1ab*, and S genes of SARS-CoV-2 were detected by qRT-PCR.

Enrichment methods and results

Enrichment of SARS-CoV-2 VLPs from VTM and CCM for protein analysis

For virus enrichment and detection of SARS-CoV-2 nucleocapsid protein N (50 kDa) by WB, VLPs were spiked into VTM or CCM, followed by a 10-minute enrichment using Dynabeads Intact Virus Enrichment beads. The enrichment was performed manually or automated using the KingFisher Flex system (Figure 2).

The WB analysis demonstrated similar enrichment efficiency with the manual and automated methods, as determined by the relative intensity of the N protein isolated from both VTM and CCM (Figure 3). This demonstrated that SARS-CoV-2 VLPs can be isolated quickly and efficiently with Dynabeads Intact Virus Enrichment beads.

Enrichment of heat-inactivated SARS-CoV-2 virus from VTM, CCM, and wastewater for nucleic acid analysis

For enrichment and nucleic acid (NA) detection of SARS-CoV-2 genes (N, *orf1ab*, and S) by qPCR, heat-inactivated SARS-CoV-2 virus was spiked into VTM, CCM, or wastewater followed by a 10-minute enrichment using Dynabeads Intact Virus Enrichment beads. Extraction of NA was performed using the Applied Biosystems™ MagMAX™ Viral/Pathogen II Nucleic Acid Enrichment Kit (VTM and CCM, Figures 4A and 4B) or MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit (wastewater, Figure 4C) after virus enrichment, followed by downstream analysis using the Applied Biosystems™ TaqPath™ COVID-19 Combo Kit.

The results demonstrated that the qRT-PCR sensitivity with beads-enriched VTM, CCM, and wastewater samples matched the sensitivity with the positive controls (directly extracted for nucleic acids, no bead enrichment; Figure 4)—the C_t values were within 2 cycles of the respective controls.

Enrichment of infectious SARS-CoV-2 from cell culture medium

For enrichment of contagious viruses, SARS-CoV-2 was collected from infected patients and was transferred to VTM. Vero cells were infected with the collected virus for 48 hours, and the viruses produced by the cells were released into the CCM and enriched by either centrifugation, precipitation using Invitrogen™ Intact Virus Precipitation Reagent, or using Dynabeads Intact Virus Enrichment beads. The amount of infectious viral particles produced was determined by counting the number of plaque-forming units on a monolayer of target cells after seeding the monolayer with the isolated virus (Ragon Institute of MGH, MIT, Harvard, USA). The counted plaques for each enrichment method are represented as fold increases compared to no virus enrichment (Figure 5). Both Dynabeads magnetic beads–based and precipitation-based enrichment resulted in higher yields of infectious SARS-CoV-2 when compared to enrichment by centrifugation alone (Figure 5B).

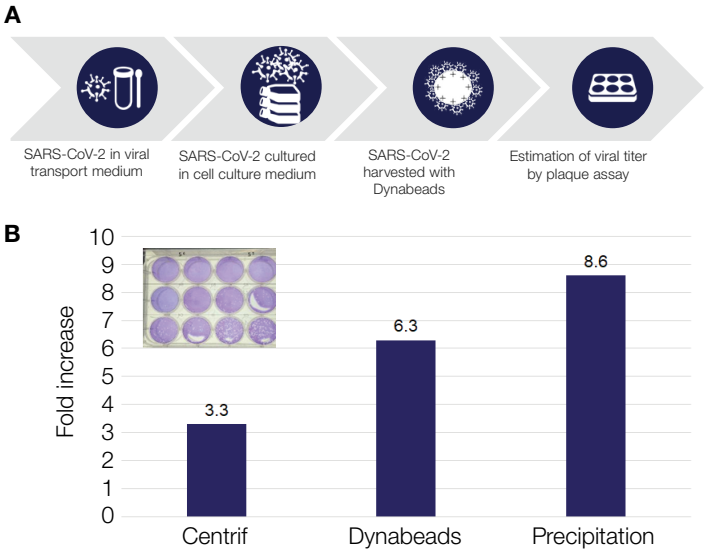

Figure 5. Enrichment of infectious SARS-CoV-2 supernatant. **(A)** Infectious SARS-CoV-2 was cultured in Vero cells and harvested after 48 hours. Viral titers after enrichment were estimated by plaque assay. **(B)** Viruses were concentrated by centrifugation, Dynabeads Intact Virus Enrichment beads, or precipitation, and seeded onto a monolayer of cells. The numbers of viruses were estimated by counting plaques (image inset).

Enrichment of viruses from wastewater for NA analysis

For enrichment and NA detection of other viruses, inactivated adenovirus, influenza A virus, norovirus, and enterovirus were spiked into 10 mL of wastewater. The viruses were enriched within 10 minutes using Dynabeads Intact Virus Enrichment beads, followed by RNA isolation using the MagMAX Microbiome Ultra kit. The results demonstrated that the Dynabeads Intact Virus Enrichment beads can isolate other negatively charged viruses besides SARS-CoV-2, and that qRT-PCR sensitivity is comparable between the enriched samples and the respective PBS controls (Figure 6).

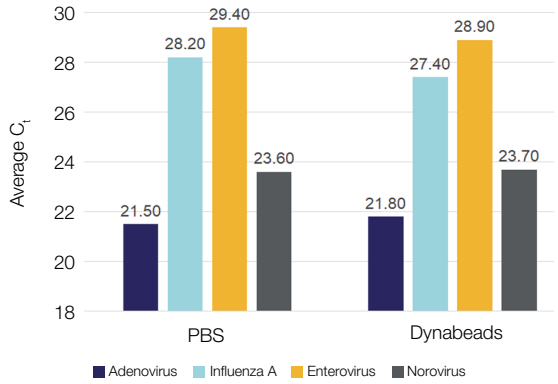

Figure 6. Enrichment of adenovirus, influenza A virus, enterovirus, and norovirus from wastewater, and detection by qRT-PCR.

Enrichment of other infectious viruses

For enrichment of other contagious viruses, Dynabeads Intact Virus Enrichment beads were added to samples containing influenza virus A, RSV, or Zika virus (ZIKV). The enrichment was assessed by western blot (Figure 7) or virus titers (Figure 8). The presence of influenza A nucleoprotein, RSV fusion protein, and Zika virus NS1 protein in samples enriched with Dynabeads Intact Virus Enrichment beads was confirmed by western blot (Figure 7). Different dilutions of bead-bound virus samples were prepared for plaque assay analysis. Plates with plaques used for calculation of virus titers are shown in Figure 8. The virus titers were 3.3 x 10^5 PFU/mL for influenza A virus, 2.6 x 10^4 PFU/mL for RSV, and 5.0 x 10^5 PFU/mL for Zika virus.

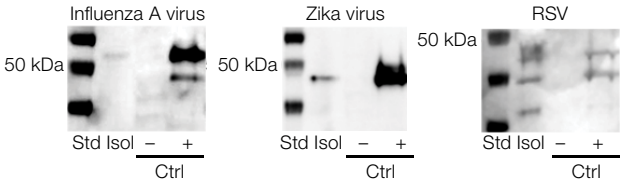

Figure 7. Enrichment of influenza A virus (H1N1), Zika virus, and RSV with Dynabeads Intact Virus Enrichment beads. Influenza A virus, Zika virus, and RSV were spiked into CCM and were captured with Dynabeads Intact Virus Enrichment beads using manual protocols, and analyzed by western blot.

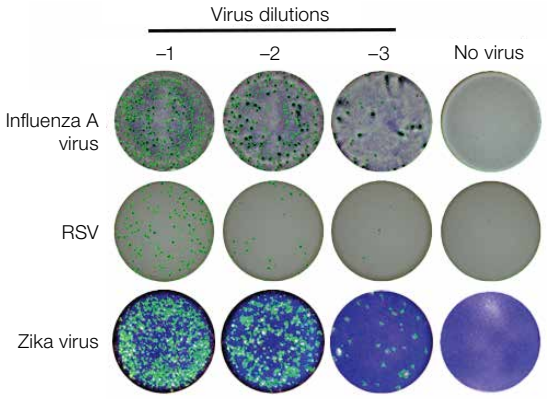

Figure 8. Enrichment of influenza A virus, RSV, and Zika virus with Dynabeads Intact Virus Enrichment beads. Viral titers after enrichment were estimated by plaque assays.

Summary

We have described an easy, rapid, and reliable bead-based method to capture a variety of viruses and VLPs by taking advantage of the strong anion-exchange principle. Virus enrichment utilized both manual and automated protocols. Enrichment was performed with Dynabeads Intact Virus Enrichment beads for multiomic analysis (qRT-PCR, western blot, and plaque assay). We demonstrated the successful isolation and release of intact infectious viruses. The automated protocol for rapid and efficient enrichment of viruses is compatible with the KingFisher Flex, Duo Prime, and Apex systems. The methods help provide investigators an easy, fast solution for the rapid enrichment of intact viruses for multiomic viral research.

Ordering information

Description	Cat. No.
Dynabeads Intact Virus Enrichment	10700D
Intact Virus Precipitation Reagent	10720D
KingFisher Flex Purification System with 96 Deep-Well Head	A32681
KingFisher 96 Deep-Well Plate, V-bottom, polypropylene (50–1,000 µL)	95040450
KingFisher 96 Tip Comb for Deep-Well Magnets	97002534
BindIt 4.0 Software (Dynabeads Intact Virus Enrichment-Flex script for download)	See 10700D
DynaMag-2 Magnet	12321D
HulaMixer Sample Mixer	15920D
4X Bolt LDS Sample Buffer	B0007
10X Bolt Sample Reducing Agent	B0004
Bolt 4–12%, Bis-Tris, 1.0 mm, Mini Protein Gel, 10-well	NW04120BOX
iBlot 2 Gel Transfer Device	IB2101
iBind Western System	SLF1000
Goat Anti–Mouse IgG1 Cross-Adsorbed Secondary Antibody, HRP	A10551
SARS/SARS-CoV-2 Coronavirus Nucleocapsid Monoclonal Antibody	MA5-29981

Learn more at **thermofisher.com/virusenrichment**