Rapid bead-based isolation of SARS-CoV-2 for multiomic viral research

Optimizing with Dynabeads Intact Virus Enrichment

Keywords

Dynabeads, KingFisher Flex, KingFisher Duo Prime, KingFisher Apex, automation, coronavirus enrichment, heat-inactivated SARS-CoV-2, virus-like particles, western blot, qPCR, viral transport medium, cell culture medium, wastewater, adenovirus, flu A virus, enterovirus, norovirus, plaque assay

In this application note, we show:

- A solution for the rapid enrichment of intact virus for multiomic viral research
- How isolation by magnetic bead–based enrichment, including from very dilute samples, can provide greater yields of intact virus essential for SARS-CoV-2 research
- The flexibility of manual handling or a simple automated workflow that enables high-throughput enrichment of many samples simultaneously
- A simple, fast, and reliable method for the isolation of intact SARS-CoV-2 virus particles for manual or automated handling

Introduction

Virus enrichment is an essential method for obtaining sufficient virus quantities often required to enable, for example, understanding of their life cycles and their 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 can be harvested from the cell culture supernatant. As viruses are in the nanometer size range and are present in variable quantities, determining an enrichment strategy may be challenging. Virus enrichment can be a tedious and difficult process, and may result in insufficient virus 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 yield. A rapid, simple, reliable, and cost-effective method is needed to concentrate intact viruses from various sample types, including cell culture medium, for manual or automated handling.

Fast and simple virus enrichment

Here we describe a manual workflow that takes less than 15 minutes to enrich intact virus from dilute cell culture and virus transport media (VTM), with an optional step to release the virus from beads in an additional ~10 minutes. This short and simple enrichment approach reduces the risk of lower yield and of affecting the integrity and infectivity of the virus. One of the key features of Invitrogen™ Dynabeads™ magnetic beads used 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 therefore fast protocols (Figure 1). Here we take advantage of the negative charge of the virus in combination with the rapid binding kinetics provided by Dynabeads magnetic beads. Instead of targeting a surface viral marker with an antibody, positively charged Invitrogen™ Dynabeads™ Intact Virus Enrichment beads bind to negatively charged viruses, other negatively charged vesicles (e.g., exosomes), or proteins within 10 minutes (Figure 1). Following capture, the virus can be released from the beads into the cell culture medium in another 10 minutes by adding an anion with a stronger relative affinity than the virus (Figures 2,3). This short and easy enrichment approach can be simplified even further by using the Thermo Scientific™ KingFisher™ Purification System (Figure 4). Here we also describe an automated enrichment method that allows larger numbers of samples to be processed with high reproducibility, reduced hands-on time, and minimal error rates, within 20 minutes. The enriched virus 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" [1].

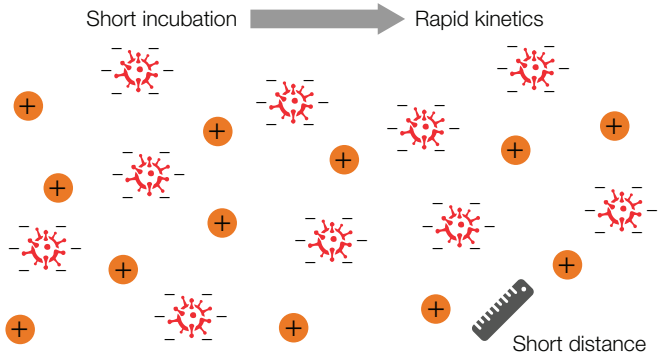

Figure 1. Binding kinetics. The positively charged Dynabeads Intact Virus Enrichment beads are in close proximity to the negatively charged SARS-CoV-2 virus, resulting in rapid binding kinetics and a fast enrichment protocol.

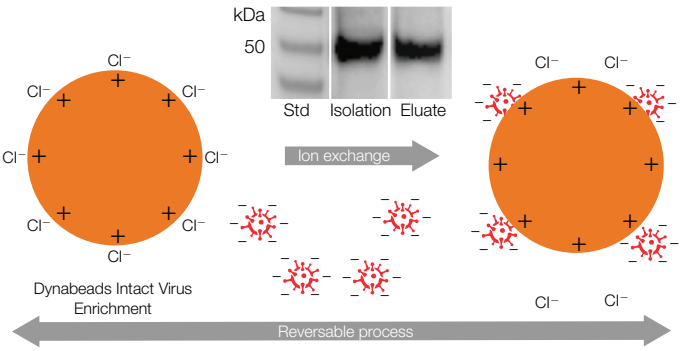

Figure 2. Principle of strong anion exchange. For enrichment of negatively charged viruses such as SARS-CoV-2, positively charged Dynabeads Intact Virus Enrichment beads protected with Cl^- ions are used. Virus particles added to the Dynabeads Intact Virus Enrichment beads will replace the Cl^- ions and bind to the bead surface. For virus particle release, an anion with higher relative affinity can be added to replace the virus and thus release the virus 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, was used.

Add Dynabeads Intact Virus Enrichment beads to the cell culture sample containing virus particles

Mix and incubate for 10 min, place tube on the magnet for 1 min, and discard the supernatant

Wash the bead–virus complexes for 2 min

Optional: add release buffer and incubate for 10 min, place tube on the magnet for 1 min, and collect the supernatant

Figure 3. Overview of the manual workflow.

Prepare KingFisher plates with Dynabeads magnetic beads, virus sample, and buffers

Load plates into the KingFisher instrument according to the protocol; press "Start"

Collect sample (virus on Dynabeads Intact Virus Enrichment beads, or released virus)

Figure 4. Overview of the automated workflow.

Overview of manual protocol and automated protocols

Both protocols use the Thermo Scientific™ KingFisher™ Flex instrument. However, the Thermo Scientific™ KingFisher™ Duo Prime or Apex instrument may also be used.

Here we illustrate the enrichment workflow using SARS-CoV-2 samples collected from infectious SARS-CoV-2 patients (Ragon Institute of MGH, MIT, Harvard, USA); or samples of viral transport medium (VTM), cell culture medium (CCM), or wastewater spiked with heat-inactivated SARS-CoV-2, SARS-CoV-2 virus-like particles (VLPs), or other viruses (adenovirus, influenza A virus, norovirus, and enterovirus). Enrichment was performed with Dynabeads Intact Virus Enrichment beads using either a manual or automated protocol to generate multiomic data (qPCR, western blot (WB), or plaque assay).

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 (Figure 5), followed by a 10-minute enrichment using Dynabeads Intact Virus Enrichment beads (optimized for SARS-CoV-2). The enrichment was performed manually or automated using the KingFisher Flex system.

The WB analysis demonstrated similar enrichment efficiency using either the manual or automated method, as determined by relative intensity of the N protein for both VTM and CCM. 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 NAs was performed using the Applied Biosystems™ MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Enrichment Kit (VTM and CCM) or MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit (wastewater) after virus enrichment, followed by downstream analysis using the Applied Biosystems™ TaqPath™ COVID-19 Combo Kit.

The results demonstrated that the qRT-PCR performance of the beads matched the sensitivity of the qRT-PCR performance of the MagMAX Viral/Pathogen II kit used as a positive control for VTM, CCM, and wastewater (Figure 6)—C_t values were within 2 cycles compared to the control.

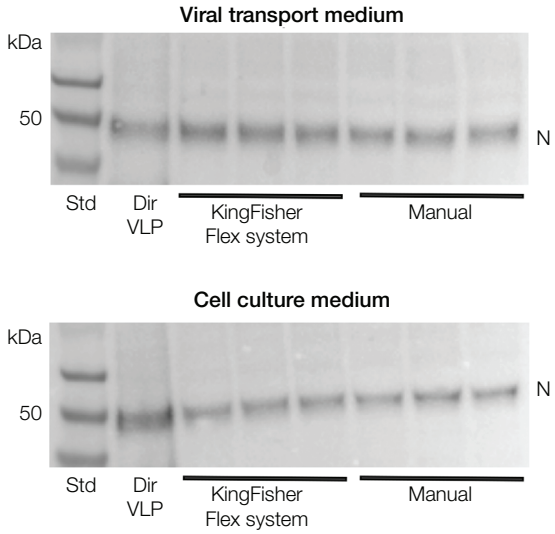

Figure 5. Enrichment of SARS-CoV-2 virus-like particles (VLPs) with Dynabeads Intact Virus Enrichment beads. VLPs were spiked into **(A)** VTM and **(B)** CCM, and were captured with Dynabeads Intact Virus Enrichment beads using manual and automated protocols. For both, SARS-CoV-2 nucleocapsid protein (N) was detected by WB.

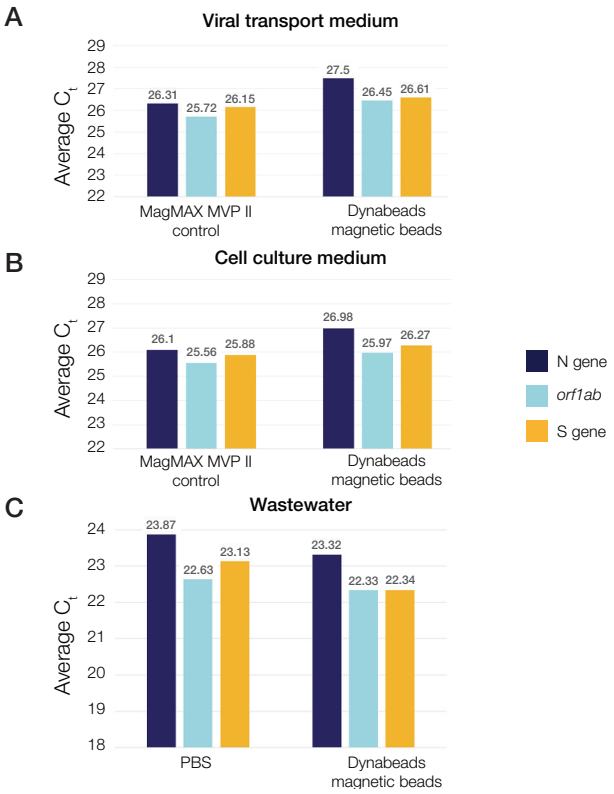

Figure 6. Enrichment of heat-inactivated SARS-CoV-2 virus from (A) VTM, (B) CCM, and (C) wastewater. The N, *orf1ab*, and S genes of SARS-CoV-2 were detected by qRT-PCR.

Enrichment of other 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. The enrichment efficiency matched the sensitivity of the PBS control (Figure 7).

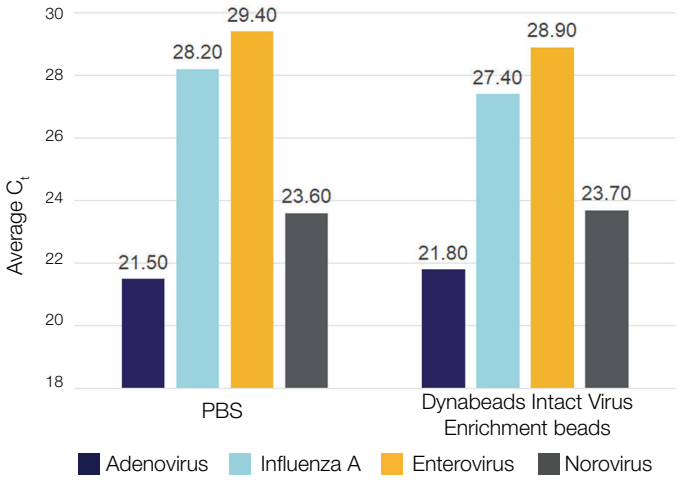

Figure 7. Enrichment of adenovirus, influenza A virus, enterovirus, and norovirus from wastewater, and detection by qRT-PCR.

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 isolated virus to the monolayer (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 8). 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 8B).

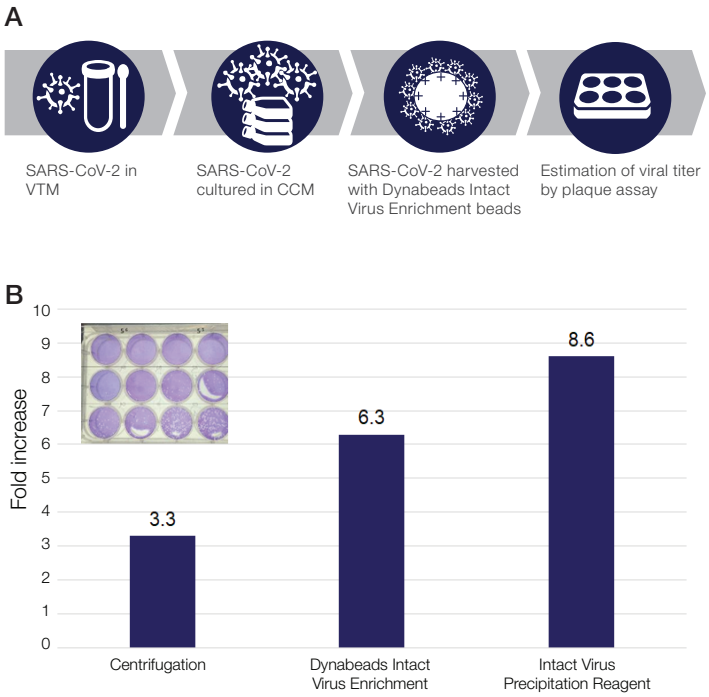

Figure 8. Enrichment of infectious SARS-CoV-2 supernatant. (A) Infectious SARS-CoV-2 was cultured in Vero cells and harvested after 48 hours. Viral titers after enrichment were estimated by plaque assay. **(B)** Viruses were concentrated by centrifugation, Dynabeads Intact Virus Enrichment beads, or precipitation.

Summary

In this application note, we have described a simple, rapid, and reliable bead-based method to capture SARS-CoV-2 virus and VLPs based upon the strong anion-exchange principle, utilizing both mahual and automated protocols. Enrichment was performed with Dynabeads Intact Virus Enrichment beads for multiomic analysis (qPCR, WB, and plaque assay). We demonstrated the successful isolation of intact virus collected from individuals infected with SARS-CoV-2 and spiked into viral transport medium (VTM) and cell culture medium (CCM). Results showed that the sensitivity in detection and yield of the virus were as expected as compared to the control. Finally, we showed that both enrichment methods using the Dynabeads Intact Virus Enrichment beads resulted in a higher yield of intact infectious SARS-CoV-2 compared to centrifugation-only methods. The beads and protocols can also be used to isolate and analyze other negatively charged viruses or vesicles. The automated protocol for rapid and efficient enrichment of viruses is compatible with the KingFisher Duo Prime, Flex, and Apex systems. Dynabeads Intact Virus Enrichment beads are found to be suitable for manual or automated enrichment of SARS-CoV-2 from VTM, CCM, and wastewater. The methods described here provide investigators a simple, fast solution for the rapid enrichment of intact virus 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

Reference

1. Dynabeads™ Intact Virus Enrichment (optimized for SARS-CoV-2). https://assets.thermofisher.com/TFS- Assets/LSG/manuals/MAN0019858_dynabeads_intact_virus_enrichment_UG.pdf

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