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Rapid enrichment of SARS-CoV-2 for multiomics analysis

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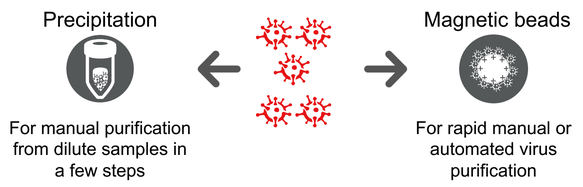
Abstract

Enrichment of viruses is essential in viral research to enhance understanding of the virus, its life cycle, and pathogenesis. 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 at the end of the viral life cycle. The released viral particles can be harvested from the cell culture supernatant. Since viruses are in the nm size range and present in variable quantities, viral enrichment can be challenging. A range of isolation and enrichment methods including ultracentrifugation and filtration are used; methods that require expensive instruments are time consuming or may result in low yield. Here we describe two short and simple methods for SARS-CoV-2 enrichment: (1) precipitation for manual purification from dilute samples in only a few steps and (2) rapid manual or automated virus purification from dilute samples using Invitrogen™ Dynabeads™ magnetic beads.

Virus enrichment



Introduction

A short and simple virus isolation approach reduces the risk of low yield and possibly affecting the integrity and infectivity of the virus. Two different virus enrichment strategies are demonstrated here by using either a precipitation reagent or Dynabeads magnetic beads. Infectious virus, inactivated virus, and virus-like particles (VLPs) can be successfully enriched using either of the methods. By tying up water molecules, Invitrogen™ Intact Virus Precipitation Reagent forces less-soluble components such as viruses out of solution, allowing them to be collected by short, low-speed centrifugation. The recovered viruses are fully intact, have a high degree of purity, and are ready for biological studies or endpoint analysis. In the enrichment approach using Dynabeads magnetic beads, the rapid binding kinetics translates into short incubation times, resulting in very fast protocols. Here we take advantage of the negatively charged virus binding to the positively charged Invitrogen™ Dynabeads™ Intact Virus Enrichment beads for rapid enrichment. Release of the virus after capture can be done by adding an anion with stronger relative affinity than the virus, releasing the virus into the cell culture medium within 10 min. This short and simple enrichment approach can be simplified further by using the Thermo Scientific™ KingFisher™ Sample Purification System.

Binding kinetics.

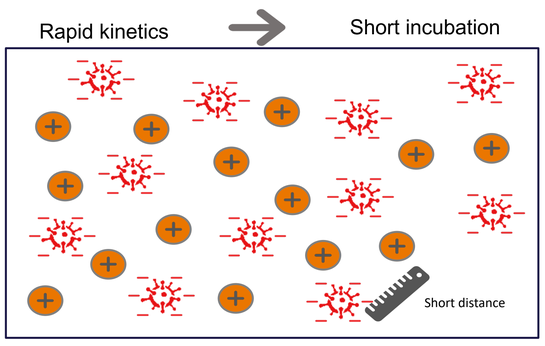


Figure 1. One of the key features of Dynabeads magnetic beads used in any enrichment protocol is the rapid binding kinetics. The close proximity of the beads to the targets in the solution translates directly into short incubation times, and thus, very fast protocols. The positively charged Dynabeads Intact Virus Enrichment beads are in close proximity to the negatively charged SARS-CoV-2, resulting in a rapid 10 min enrichment protocol.

Strong anion exchange.

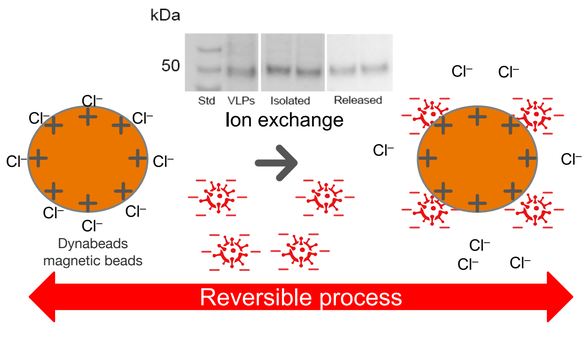
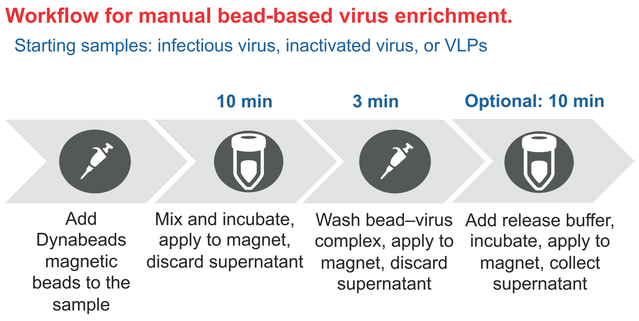


Figure 2. For enrichment of negatively charged viruses, such as SARS-CoV-2, a positively charged Dynabeads Intact Virus Enrichment product protected with Cl^- ions is used. Virus added to the beads will replace the Cl^- ions and bind to the bead surface. For viral release, an anion with higher relative affinity can be added to replace the virus and thus release the virus into the medium.

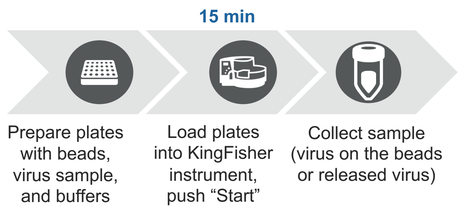
Results

Bead-based SARS-CoV-2 enrichment

Workflow for manual bead-based virus enrichment.
Starting samples: infectious virus, inactivated virus, or VLPs



Workflow for automated bead-based virus enrichment.
Starting samples: infectious virus, inactivated virus, or VLPs



Enrichment of SARS-CoV-2 VLPs.

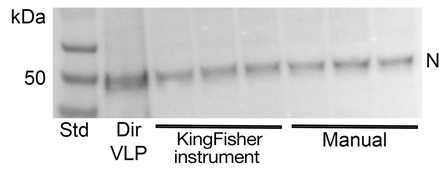


Figure 5. Enrichment of SARS-CoV-2 VLPs from cell culture medium. Manual or automated enrichment of VLPs spiked into cell culture medium using Dynabeads Intact Virus Enrichment beads. SARS-CoV-2 nucleocapsid protein (N) was detected by western blot analysis. Similar results were obtained from viral transport medium (data not shown).

Enrichment of heat-inactivated SARS-CoV-2.

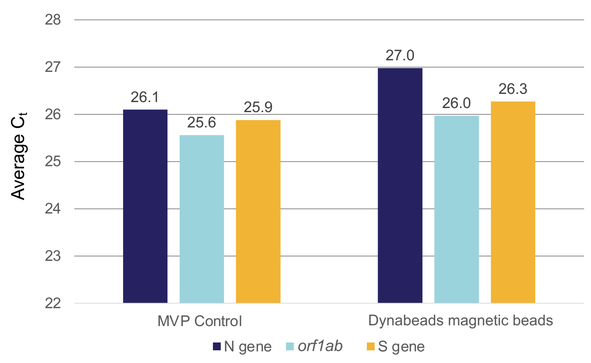


Figure 6. Enrichment of heat-inactivated SARS-CoV-2 from cell culture medium. SARS-CoV-2 was detected by RT-qPCR of N gene, *orf1ab*, and S gene. Similar results were obtained from viral transport medium and wastewater (data not shown).

Enrichment of other viruses.

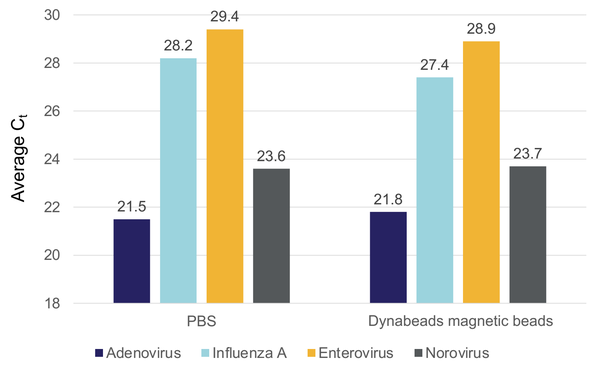
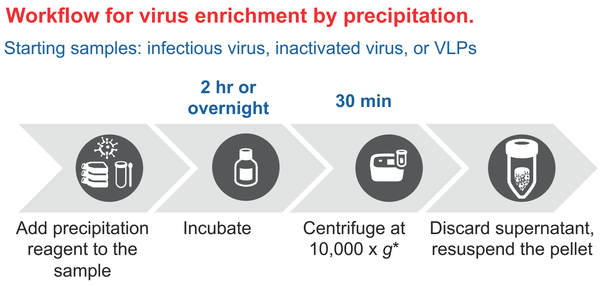


Figure 7. Enrichment of adenovirus, influenza A virus, enterovirus, and norovirus was successfully performed from wastewater samples, and the viruses were subsequently detected by RT-qPCR.

Precipitation-based SARS-CoV-2 enrichment

Workflow for virus enrichment by precipitation.
Starting samples: infectious virus, inactivated virus, or VLPs



* For qPCR, use 3,200 x *g*.

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

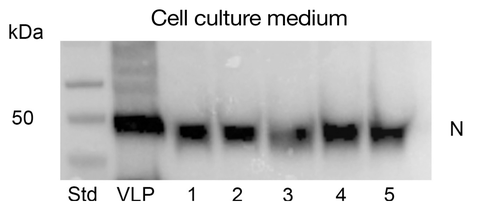


Figure 9. VLPs spiked into 5 samples of cell culture medium followed by precipitation with Intact Virus Precipitation Reagent. After precipitation, the samples were subjected to SDS-PAGE and western blot analysis. The presence of VLPs is demonstrated by the 50 kDa band for the nucleocapsid protein N.

Enrichment of SARS-CoV-2 from viral transport medium.

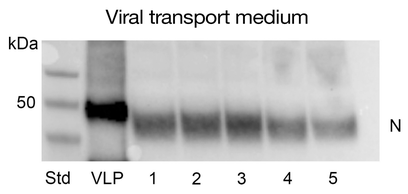


Figure 10. VLPs spiked into 5 samples of viral transport medium followed by precipitation with Intact Virus Precipitation Reagent. After precipitation, the samples were subjected to SDS-PAGE and western blot analysis. The presence of VLPs is demonstrated by the 50 kDa band for the nucleocapsid protein N.

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

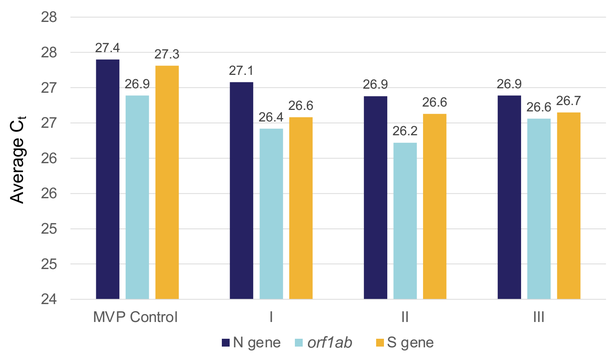


Figure 11. Heat-inactivated virus spiked into 3 samples of cell culture medium followed by precipitation with Intact Virus Precipitation Reagent. SARS-CoV-2 was detected by RT-qPCR of N gene, *orf1ab*, and S gene.

Enrichment of SARS-CoV-2 from viral transport medium.

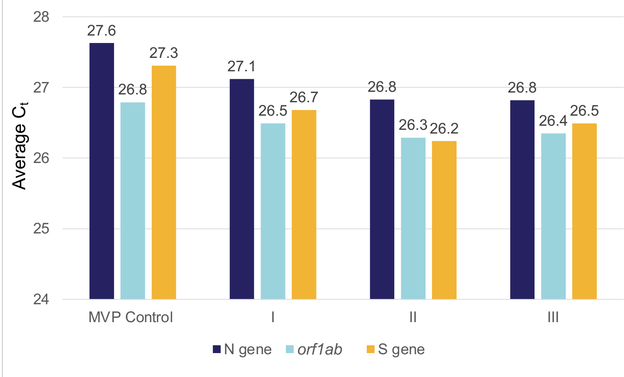
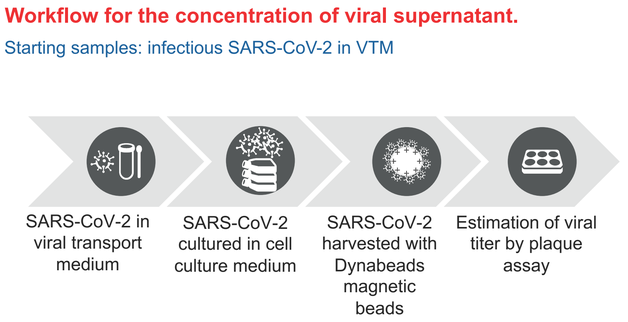


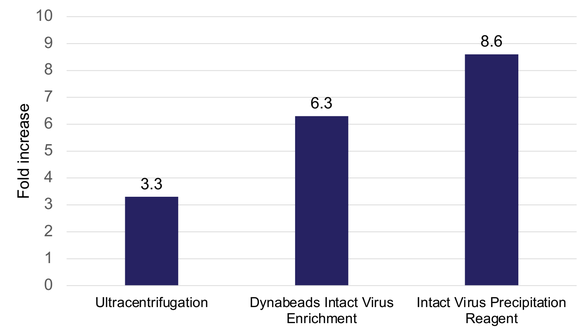
Figure 12. Heat-inactivated virus spiked into 3 samples of viral transport medium followed by precipitation with Intact Virus Precipitation Reagent. SARS-CoV-2 was detected by RT-qPCR of N gene, *orf1ab*, and S gene.

Infectious SARS-CoV-2 enrichment

Workflow for the concentration of viral supernatant.
Starting samples: infectious SARS-CoV-2 in VTM



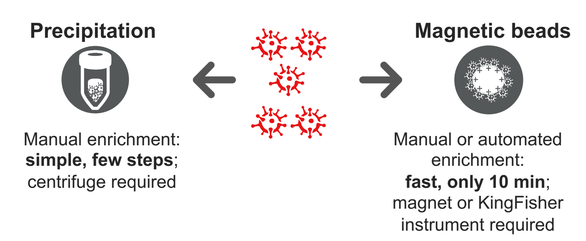
Concentration of viral supernatant.



Figures 13 and 14. Concentration of infectious viral supernatant. Infectious SARS-CoV-2 was cultured in Vero cells in cell culture medium for 48 hours followed by enrichment by ultracentrifugation, precipitation, or with Dynabeads magnetic beads. Fold increase of viral titers after enrichment was estimated by plaque assay (assay performed at Ragon Institute of MGH, MIT, and Harvard).

Conclusions

We have developed two methods for rapid SARS-CoV-2 enrichment: (1) precipitation for manual purification from dilute samples with only a few steps and (2) rapid manual or automated virus purification from dilute samples using Dynabeads magnetic beads. The products are suitable for enrichment of SARS-CoV-2 from viral transport medium, cell culture medium, and wastewater samples for downstream protein and nucleic acid analysis. SARS-CoV-2 enriched using the Dynabeads magnetic bead–based method can easily be released from the beads in 10 min. The methods can also be used to isolate and analyze other negatively charged viruses and vesicles (e.g., exosomes) and VLPs. The automated protocol for rapid and efficient enrichment of viruses is compatible with the KingFisher Duo Prime, Flex, and Apex instruments.



Materials

Intact Virus Precipitation Reagent (Cat. No. 10720D), Dynabeads Intact Virus Enrichment (Cat. No. 10700D), Invitrogen™ DynaMag™-2 Magnet (Cat. No. 12321D), Invitrogen™ HulaMixer™ Sample Mixer (Cat. No. 15920D), Applied Biosystems™ MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Cat. No. A42352, A48383), Applied Biosystems™ TaqPath™ COVID-19 Combo Kit (Cat. No. A47814), Invitrogen™ 4X Bolt™ LDS Sample Buffer (Cat. No. B0007), Invitrogen™ 10X Bolt™ Sample Reducing Agent (Cat. No. B0004), Invitrogen™ Bolt™ 4 to 12%, Bis-Tris, 1.0 mm, Mini Protein Gel (Cat. No. NW04120BOX), Invitrogen™ iBlot™ 2 Gel Transfer Device (Cat. No. IB21001), Invitrogen™ iBind™ Western System (Cat. No. SLF1000), Invitrogen™ Goat Anti-Mouse IgG1 Cross-Adsorbed Secondary Antibody, HRP (Cat. No. A10551), Invitrogen™ SARS/SARS-CoV-2 Nucleocapsid Monoclonal Antibody (Cat. No. MA5-29981)

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