Virus enrichment

Rapid precipitation-based enrichment of SARS-CoV-2 for multiomics viral research

Optimization using the with Intact Virus Precipitation Reagent

In this application note, we show:

- A simple, fast, and reliable method for the isolation of SARS-CoV-2 particles
- A solution for the rapid enrichment of intact virus for multiomic viral research
- How isolation of viruses, including from very dilute samples, by precipitation-based enrichment can provide greater yields of intact virus essential for SARS-CoV-2 research

Introduction

Enrichment of viruses, including SARS-CoV-2, is essential to enhance understanding of viral life cycles and pathogenesis. Multiomic study of viruses requires isolation of intact virus particles, often from large volumes and very dilute samples. To address these issues, stocks of virus can be made by inoculating cell cultures with a seed virus. The infected cells will release new viral particles into the cell culture media (CCM) at the end of the viral life cycle. The released viral particles can then 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 that may result in insufficient virus yield. Low virus titers can introduce artificial variants or a bias in gene sequences. A range of methods, including different forms of ultracentrifugation, precipitation, and filtration, is used; these methods require expensive instruments, are very time consuming, and may result in low yields. A rapid, simple, and reliable cost-effective method is needed for the concentration of intact viruses from various sample media, including CCM.

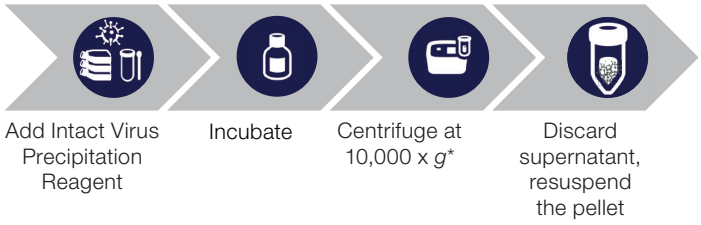
Fast and simple virus enrichment

Here we describe a three-step workflow that takes less than 3 hours to perform. This short and simple enrichment approach reduces the risks of lower yield and affecting the integrity and infectivity of the virus. By tying up water molecules, the 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 (Figure 1). We demonstrate that the recovered viruses are fully intact, have a relatively high degree of purity, and are ready for either biological studies or endpoint analysis. For details on the protocol, see "Intact Virus Precipitation Reagent" [1].

Figure 1. Three-step workflow. The procedure is performed in three simple steps and takes less than 3 hours to complete with minimal hands-on time.

Overview of protocols

An overview of the precipitation workflow is shown in Figure 2.



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

Figure 2. Precipitation workflow.

invitrogen

Prepare sample

1. Harvest CCM or virus transport media (VTM).
2. Centrifuge the media at 3,200 x *g* at 2–8°C for 15 minutes to remove cells and debris.
3. Gently transfer the supernatant to a new tube without disturbing the pellet.

Precipitate intact virus

1. Transfer the required volume of cell-free media to a new tube and add 0.5 volumes of Intact Virus Precipitation Reagent (ratio 1:2 of precipitation reagent to virus-containing media). The protocol is directly scalable and can be scaled up or down accordingly.
2. Vortex or pipet the solution up and down until it is homogeneous.
3. Incubate the sample at 2–8°C for 2 hours (or overnight at 2–8°C).
4. Centrifuge the sample at 10,000 x *g* at 2–8°C for 30 minutes. If the intended downstream use is qPCR, reduce the centrifugation speed to 3,200 x *g*.
5. Aspirate and discard the supernatant.
6. Resuspend the pellet in 1X PBS or a similar buffer. The user will determine the volume of buffer to use based upon the pellet size.

Once the pellet is resuspended, the virus is ready for downstream analysis, ranging from functional studies to endpoint analysis of RNA and protein cargo. The enriched viral particles can be used directly in qPCR analysis, or RNA can be purified with Applied Biosystems™ MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kits.

The enrichment protocol works reliably for infectious SARS-CoV-2, inactivated SARS-CoV-2, and SARS-CoV-2 virus-like particles (VLPs). Note that VLPs do not contain nucleic acids (NAs), and thus can only be used for proteomic studies.

Here we illustrate the enrichment workflow using SARS-CoV-2 samples collected from infectious SARS-CoV-2 individuals [2], or from spiking heat-inactivated SARS-CoV-2 or SARS-CoV-2 VLPs into VTM or CCM. Enrichment was performed with Intact Virus Precipitation Reagent for multiomics data generation (qPCR, western blot (WB), or plaque assay).

Enrichment methods and results

Enrichment of heat-inactivated SARS-CoV-2 virus from VTM and CCM for NA analysis

For enrichment and NA detection of SARS-CoV-2 genes (nucleocapsid protein (N), *orf1ab*, and spike protein (S)) by qPCR, inactivated SARS-CoV-2 spiked into VTM or CCM was mixed with Intact Virus Precipitation Reagent, followed by a 2-hour incubation at 2–8°C (alternatively, overnight). After incubation, the sample was centrifuged at 3,200 x *g* at 2–8°C for 30 minutes. Extraction of NAs was performed using the Applied Biosystems™ MagMAX™ Viral/Pathogen II Nucleic Acid Enrichment Kit, followed by downstream PCR analysis with the Applied Biosystems™ TaqPath™ COVID-19 Combo Kit. SARS-CoV-2 was detected by qPCR of the N, *orf1ab*, and S genes. As expected, the results demonstrated that the performance matched the sensitivity of the MagMAX kit control for CCM (Figure 3A) and VTM (Figure 3B) (C_t values were within ±2 units compared to the control).

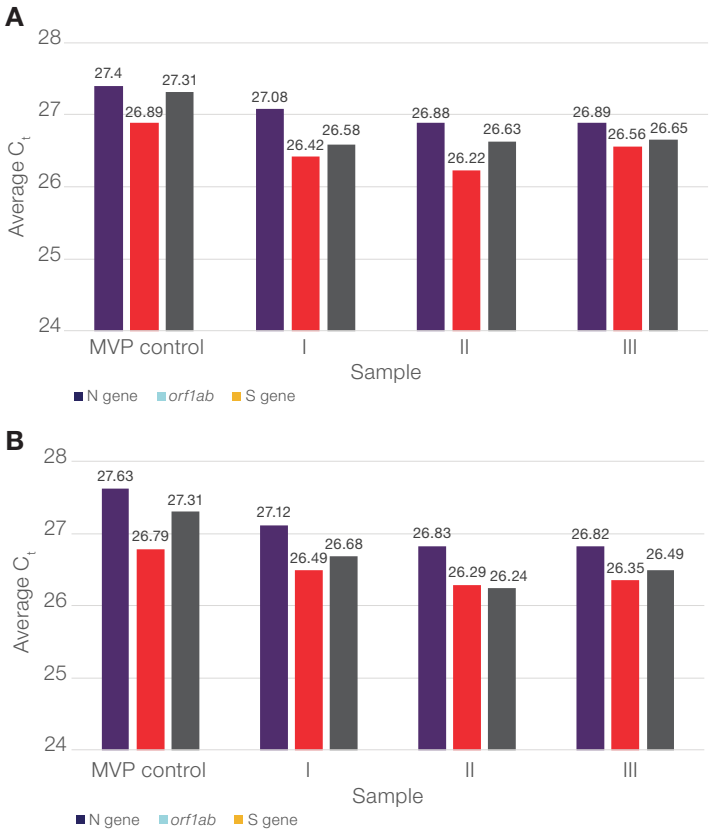


Figure 3. Heat-inactivated SARS-CoV-2 was spiked into 3 samples of (A) CCM or (B) VTM, followed by precipitation with the Intact Virus Precipitation Reagent. The N, *orf1ab*, and S genes of SARS-CoV-2 were detected by qPCR. I, II and III represent three parallel precipitations. Results are presented as averaged C_t values.

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

For virus enrichment and detection of the SARS-CoV-2 nucleocapsid protein N (50 kDa) by WB, VLPs were spiked into CCM or VTM and mixed with Intact Virus Precipitation Reagent, followed by a 2-hour incubation at 2–8°C (alternatively, overnight at 2–8°C). After incubation, the samples were subjected to centrifugation at 10,000 x *g* at 2–8°C for 30 minutes. The enriched SARS-CoV-2 samples were separated by SDS-PAGE. Subsequently, protein transfer was performed using an Invitrogen™ iBlot2™ Gel Transfer Device. Immunolabeling was performed using an Invitrogen™ iBind™ Western Device. In Figures 4A and 4B, the presence of VLPs is demonstrated by the detection of the 50 kDa N protein from CCM and VTM samples, respectively. The WB analysis also demonstrated similar enrichment efficiency for all parallel samples in either CCM or VTM, as evident by the detection of the N protein at similar densities across the 5 samples. This illustrates that SARS-CoV-2 VLPs can be enriched rapidly and efficiently using the Intact Virus Precipitation Reagent.

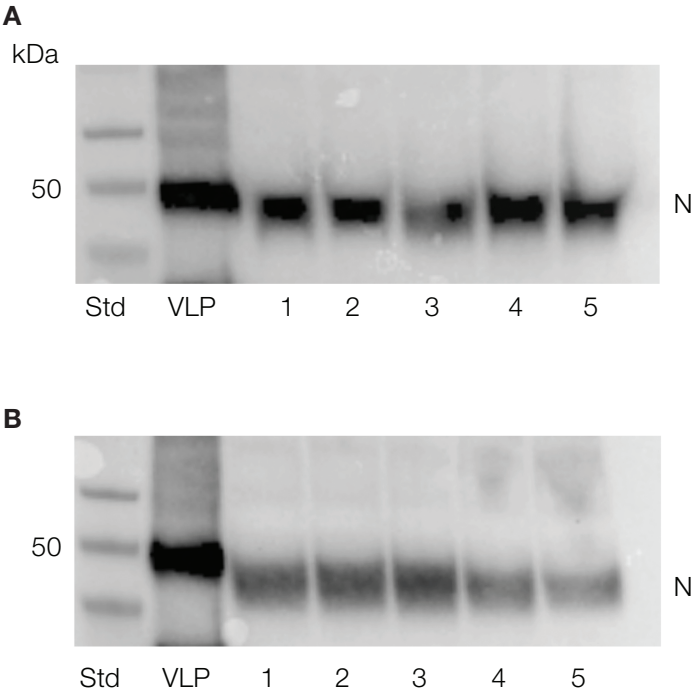


Figure 4. Enrichment of SARS-CoV-2 VLPs from CCM and VTM. The VLPs were spiked into 5 samples of **(A)** CCM and **(B)** VTM, followed by precipitation with Intact Virus Precipitation Reagent. After precipitation, the samples were subjected to SDS-PAGE and WB. The presence of VLPs was demonstrated by detection of protein N.

Enrichment of infectious SARS-CoV-2 from CCM

For enrichment of infectious virus, SARS-CoV-2 was collected from infected individuals and transferred to VTM. Vero cells were infected with the virus for 48 hours, and the virus released into CCM was enriched by either centrifugation, precipitation using Intact Virus Precipitation Reagent, or bead-based enrichment using Invitrogen™ Dynabeads™ Intact Virus Enrichment beads. The amount of infectious viral particles was determined by a plaque assay, comparing the number of plaque-forming units on a monolayer of target cells after seeding isolated virus to the monolayer (Figure 5A) [2]. Both Dynabeads magnetic beads– and precipitation-based enrichment resulted in a higher yield of infectious SARS-CoV-2 when compared to enrichment by centrifugation alone (Figure 5B).

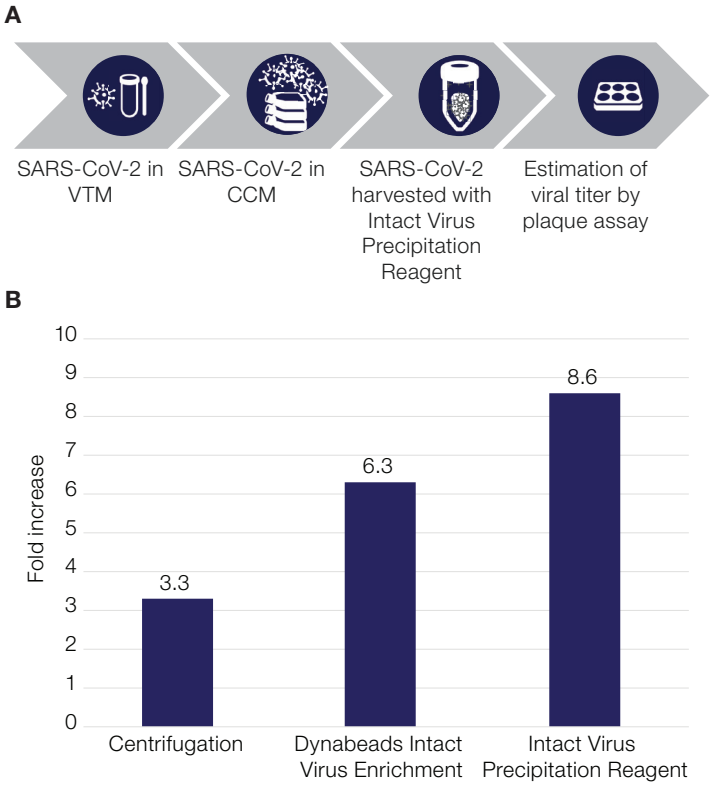


Figure 5. Enrichment of SARS-CoV-2 supernatant. (A) Infectious SARS-CoV-2 was cultured in Vero cells and harvested after 48 hours. **(B)** Virus was concentrated by centrifugation, Dynabeads magnetic beads, or precipitation [2].

Summary

Here we have described a simple, rapid, and reliable enrichment method based upon precipitation for manual purification of SARS-CoV-2 from dilute samples. Enrichment was performed using the Intact Virus Precipitation Reagent for multiomic analysis (qPCR, WB, and plaque assay). We demonstrated successful isolation of intact virus, collected from individuals infected with SARS-CoV-2, spiked into VTM or CCM. The results showed that the sensitivity in detection and yield of the virus were as expected when compared to controls. Finally, we showed that both Dynabeads magnetic beads– and precipitation-based enrichment resulted in a higher yield of intact infectious SARS-CoV-2 compared to the centrifugation-only method. The Intact Virus Precipitation Reagent is suitable for enrichment of SARS-CoV-2 from VTM or CCM for downstream protein and NA analysis. The method can also be used to isolate and analyze other viruses or vesicles, such as exosomes. The methods described here provide investigators a simple, fast solution for the rapid enrichment of intact virus for multiomic viral research.

Ordering information

Product	Cat. No.
Intact Virus Precipitation Reagent	10720D
MagMAX Viral/Pathogen Nucleic Acid Isolation Kit	A42352
MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit	A48383
Dynabeads Intact Virus Enrichment	10700D
4X Bolt LDS Sample Buffer	B0007
10X Bolt Sample Reducing Agent	B0004
Bolt 4 to 12%, Bis-Tris, 1.0 mm, Mini Protein Gel, 10-well	NW04120BOX
iBlot 2 Gel Transfer Device	IB21001
iBind Western Device	SLF1000
Goat anti-Mouse IgG1 Cross-Adsorbed Secondary Antibody, HRP	A10551
SARS/SARS-CoV-2 Nucleocapsid Monoclonal Antibody	MA5-29981

References

1. Intact virus precipitation reagent (optimized for SARS-CoV-2). https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0019857_intact_virus_ppt_reagent_PI.pdf.

2. Ragon Institute of MGH, MIT, Harvard, USA.

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