Intact Virus Precipitation Reagent

Catalog Number 10720D

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

Biological study of viruses often requires isolation of intact virus particles from very dilute samples. Current isolation methods such as ultracentrifugation are tedious and difficult. The Intact Virus Precipitation Reagent provides a simple, fast and reliable method for concentration of intact viruses from various samples such as cell culture media and liquid biopsies. By tying up water molecules, this reagent forces less-soluble components (i.e., viral particles) out of solution, allowing them to be collected after brief, low-speed centrifugation. The procedure is performed in three simple steps and takes less than 3 hours to perform.

The provided user protocol is optimized for SARS-CoV-2 but have been successfully used with other enveloped viruses such as Infuenza A-, Ebola-, Zika-, and respiratory syncytial virus (RSV). Additionally, the virus enrichment process can be automated using the Dynabeads™ Intact Virus Enrichment magnetic beads in combination with any of the KingFisher™ sample purification instruments.

Contents and storage

The Intact Virus Precipitation Reagent processes up to 100 mL of cell culture media.

Components	Amount	Storage
Intact Virus Precipitation Reagent	50 mL	2–8°C

General guidelines

- The enrichment protocol works reliably for infectious viruses, inactivated viruses, and virus-like particles (VLP's). Note that VLP's do not contain nucleic acids, and thus can only be used for proteomic studies.
- Fetal bovine serum (FBS) contains high levels of naturally occurring exosomes, which will be co-purified with the viral particles due to near identical size and properties. If this is a concern when virus is enriched from cell culture media containing FBS, use exosome-depleted FBS.

Prepare sample

1. Harvest cell culture media or virus containing sample.
2. Centrifuge at 3,200 × *g* at 2–8°C for 15 minutes to remove cells and debris.
3. Transfer the supernatant gently to a new tube without disturbing the pellet.

Precipitate intact virus

1. Transfer the required volume of cell-free media or sample to a new tube then add 0.5 volumes of the Intact Virus Precipitation Reagent (ratio 1:2 of precipitation reagent:virus-containing sample). The protocol is directly scalable and can be scaled up or down accordingly.

Sample starting volume	Precipitation Reagent volume
1 mL	500 µL
10 mL	5 mL

2. Vortex or pipette the solution up and down until there is a homogenous solution.
3. Incubate samples at 2–8°C for 2 hours (or overnight, if preferred).
4. Centrifuge the sample at 10,000 × *g* at 2–8°C for 30 minutes.

   Note: Viral particles are contained in the pellet at the bottom of the tube for swing-out rotors or on the tube wall for fixed angle rotors (not visible in some cases).

   If the intended downstream use is qPCR, reduce centrifugation speed to 3,200 × *g*.
5. Aspirate and discard the supernatant.
6. Resuspend the pellet in a convenient volume of 1X PBS or similar buffer (example volumes are shown below).

Sample starting volume	Resuspension volume
1 mL	25–100 µL
10 mL	100 µL to 1 mL

7. Once the pellet is resuspended, the virus is ready for downstream analysis, ranging from functional studies to end-point analysis of RNA and protein cargo. The enriched viral particles can be used directly in qPCR analysis, or RNA can be purified with MagMAX™ RNA extraction kits.

Protein analysis by electrophoresis

When performing western blot analysis, follow the standard procedures for your electrophoresis apparatus using a wide range protein gel (e.g., Bolt™ 4 to 12% Mini Protein Gel).

Prepare sample for electrophoresis

This is a simple protocol for sample preparation prior to electrophoresis. Further optimization may be required for optimal results.

1. Resuspend the enriched virus from “Precipitate intact virus” in 30 µL of distilled water.

   Note: If your antibody requires reducing conditions reduce the volume distilled water to 26 µL, and add 4 µL 10X Bolt™ Sample Reducing Agent to the sample.

2. Add 10 µL 4X Bolt™ LDS Sample Buffer.

3. Heat for 10 minutes at 70°C.

4. Load supernatant containing isolated virus into the wells of the gel for electrophoresis.

Perform western blot

For convenience the iBlot™ 2 Gel Transfer Device can be used for efficient blotting transfer within seven minutes after electrophoresis without the need for liquid buffers. For fast and automated immunodetection, the iBind™ Western System can be used.

Related products

Product	Cat. No.
Dynabeads™ Intact Virus Enrichment[1]	10700D
Exosome-depleted FBS	A2720803
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 System	SLF1000
Goat anti-Mouse IgG_1 Cross-Adsorbed Secondary Antibody, HRP	A10551

[1] For use in manual or automated protocols with KingFisher™ instruments

Limited product warranty

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Revision history: Pub. No. MAN0019857

Revision	Date	Description
B.0	29 August 2023	The product name was edited and minor errors were corrected.
A.0	8 January 2021	New document for Intact Virus Precipitation Reagent (optimized for SARS-CoV-2).