Dynabeads™ Intact Virus Enrichment

Catalog Numbers 10700D, 10701D

Pub. No. MAN0019858 Rev. B.0

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 dilute samples. Current isolation methods such as ultracentrifugation are tedious, difficult, and cannot be automated. The Dynabeads™ Intact Virus Enrichment provides a simple, fast and reliable method for concentration of intact viruses from various samples such as cell culture media and virus transport media (VTM) for manual or automated handling. The procedure is simple, and performed in less than 30 minutes. The enriched virus particles can be released from the magnetic beads for subsequent applications, if necessary.

Dynabeads™ Intact Virus Enrichment contains highly positively charged, monosized, superparamagnetic beads that strongly bind negatively charged vesicles or molecules in the sample. This protocol provides a good starting point for enrichment of viruses such as SARS-CoV-2 and SARS-CoV-2-virus-like particles (VLP's), but has also been verified for the following viruses; Infuenza A-, Entero-, Adeno-, Noro-, Ebola, Zika-, and Respiratory Syncytial Virus (RSV). 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).

These beads also enrich for other negatively charged proteins and vesicles, such as exosomes. Contact us for information on exosome enrichment protocols.

Contents and storage

Amount [1]	Cat. No.	Cat. No.
2 mL (100 reactions)	10700D	2–8°C
10 mL (500 reactions)	10701D	

[1] Contains 40 mg/mL of 1 µm sized strong anion exchange superparamagnetic beads supplied in 30 mM sodium chloride (NaCl) and 0.05% sodium azide (NaN_3).

Required materials not supplied

- DynaMag™ Magnet (see thermofisher.com/magnets to find the most suitable for your volumes).
- Sample mixer or roller allowing tilting and rotation of tubes (e.g., HulaMixer™ Sample Mixer).
- See “Automated enrichment protocol” for additional materials.

Buffers and solutions

The following reagents are general recommendations. Alternative buffers may also be used.

Washing buffer (choose one):

- 10 mM NaCl in 20 mM triethanolamine, pH 6
- Phosphate buffered saline (PBS)

Isolation buffers (choose one):

- PBS
- Dulbecco's Phosphate Buffered Saline (DPBS)
- Hanks Balanced Salt Solution (HBSS)

Release buffers (choose one):

- 0.25 M KI in 20 mM triethanolamine, pH 6
- 0.25 M KI in 20mM triethanolamine HCl, pH 6
- 50 mM citric acid, 50 mM Na-phosphate, pH 4

General guidelines

- The protocols are optimized for SARS-CoV-2 virus and SARS-CoV-2 VLPs in cell culture media and VTM. For other sample types, further optimization may be required (e.g., bead amount and incubation time).
- Isolated virus can be released in 10 minutes using Release Buffer, but further optimization may be required (e.g., release time and salt concentration).
- Increasing the release volume can increase virus yield, whereas reducing the release volume can be used to concentrate the virus sample (e.g., for western blot).
- Perform procedures at room temperature, unless otherwise stated.

Manual enrichment protocol

This protocol provides a general procedure for enrichment of intact infectious or inactivated SARS VLP’s from cell culture media or VTM. Optimization depending on sample and virus type might be required.

- One reaction is defined as 20 µL Dynabeads™ Intact Virus Enrichment per 1 mL virus sample. Volumes can be scaled up or down proportionally as required.
- Because virus concentration vary between samples, sample volume must be optimized (e.g., 20 µL beads can be used with a sample volume ranging from 200 µL to 1 mL).
- Remove the sodium azide by washing the beads prior to virus binding (see “Wash magnetic beads”).

Table 1 Examples of total reagent volumes for virus enrichment

Virus starting volume	Bead volume	Washing Buffer
1 mL	20 µL	~1.5 mL
5 mL	100 µL	~7 mL
20 mL	400 µL	~30 mL

Wash magnetic beads

For multiple samples, the beads can be washed in one large bulk volume sufficient for all of the samples.

1. Resuspend the vial of Dynabeads™ magnetic beads (e.g., place on a roller for ~5 minutes).
2. Immediately pipette 20 µL of resuspended beads from the vial to a new tube.
3. Add 400 µL Washing Buffer to the tube, then mix thoroughly.
4. Apply to a DynaMag™ magnet for 1 minute, then remove the buffer.

Enrich for virus particles

1. Add 1 mL of virus sample into the tube containing the pre-washed beads (alternatively, add 20 µL pre-washed beads into 1 mL of sample).
2. Incubate on a roller for 10 minutes.
3. Apply to a DynaMag™ magnet for 1 minute, then remove the supernatant.
4. Add 1 mL Washing Buffer, then mix thoroughly.

   Note: Alternatively, for downstream qRT-PCR, the bead-bound virus can be directly resuspended in 1X PBS and Viral/Pathogen Kit Binding Buffer (see "Extract RNA for qRT-PCR (Automated)" on page 3).
5. Apply to a DynaMag™ magnet for 1 minute, then remove the buffer.
6. Go directly to "(Optional) Release virus particles" on page 2 or resuspend the bead-bound virus in a suitable buffer and volume for your downstream assay.

(*Optional*) Release virus particles

Start with the bead-bound virus particles from Enrich for virus particles (not resuspended). The protocol is scaled for 20 µL beads/mL virus starting sample.

1. Add 50 µL Release Buffer and mix well.
2. Incubate on a roller for 10 minutes.
3. Mix well by pipetting or vortexing.
4. Apply to a DynaMag™ magnet for 1 minute.
5. Transfer the supernatant containing the isolated virus to a new tube.

   The isolated and released virus is now ready for downstream analysis.

   Note: The sample is dissolved in high salt at the end of the procedure. For buffer exchange, use a suitable Spin Column such as the EchoLUTION Viral RNA/DNA Swab Kit Plus.

(*Optional*) Prepare sample for electrophoresis

Volumes are adapted for 1 well (~40 µL/well). Further optimization may be required for optimal results.

1. Resuspend the bead-bound virus from "Enrich for virus particles" 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. Apply to DynaMag™ magnet for 1 minute.
5. 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.

(*Optional*) Extract RNA for qRT-PCR

After virus enrichment, RNA extraction can be performed on the sample using the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit for downstream qRT-PCR. See the *MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (manual extraction) User Guide* (MAN0018072) for manual extraction instructions. For an automated procedure, see "Extract RNA for qRT-PCR" on page 3.

Automated enrichment protocol

This section provides an automated protocol for the KingFisher™ Flex and KingFisher™ Apex instruments. Other KingFisher™ instruments can also be used. Go to **https://www.thermofisher.com/order/catalog/product/10700D?SID=srch-srp-10700D** or contact Technical support for more information regarding protocols for other KingFisher™ instruments.

Required materials not supplied

- KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head
- KingFisher™ Deep-Well 96 Plate, V-bottom, polypropylene (50-1000 µL)
- KingFisher™ Flex 96 Tip Comb for Deep-Well Magnets
- BindIt™ 4.0 Software for KingFisher™ Flex and KingFisher™ Duo Prime
- BindIx™ Software for KingFisher™ Apex

See **www.thermofisher.com/automation** for alternative plates and magnetic heads (e.g., for 24-wells).

For downloading of the software and protocols, go to **www.thermofisher.com/automation**.

General guidelines

- This protocol is for KingFisher™ Flex and KingFisher™ Apex 96-deep well plates, but other suitable KingFisher plastic for 96-well heads and 24-well heads and corresponding plastic can be used as well.
- 1 reaction is defined as 10 µL beads from the original Dynabeads vial in 200 µL virus cell culture or VTM media. Optimize the volumes depending on the virus concentration (range from ~200-500 µL virus media per 10 µL beads). **Note:** Since these volumes are half of the manual protocol volumes, the automated #rxns are doubled (200 rxns per 2 mL beads, and 1000 rxns per 10 mL beads).
- It is important to resuspend the Dynabeads vial prior to starting the procedure (e.g. leave on a roller for ~5 minutes).
- If you need the virus to be released from the beads, see protocol guidelines in the manual section.
- Do not elute in a volume smaller than 30 µL/well.

Prepare plates

- Prepare 4 plates and 1 tip-comb plate for each run.
- Dilute the Dynabeads™ magnetic beads 1:10 in Washing Buffer prior to loading the beads in the wells (e.g., 10 µL beads are diluted to 100 µL per reaction which is added to plate 1).

Table 2 Plate set-up and volume requirements for KingFisher™ Apex and KingFisher™ Flex instruments

Plate position	Plate name	Reagent	Volume/well
1	Tip Comb	–	–
2	Dynabeads	Diluted Dynabeads	100 µL
3	Sample	Virus in medium	200 µL
4	Wash	Wash Buffer	300 µL
5	Isolated virus	Wash Buffer	200 µL

Table 3 Plate set-up and volume requirements for the KingFisher™ Duo Prime instrument

Plate position	Plate name	Reagent	Volume/well
A	Dynabeads	Diluted Dynabeads	100 µL
B	Sample	Virus in medium	200 µL
C	Wash	Wash Buffer	300 µL
D	Isolated virus	Wash Buffer	200 µL
H	Tip Comb	–	–

Run automated program

1. Select the appropriate KingFisher™ script on the instrument.
2. Press **Start**.
3. Open the instrument door, load the plates into the instrument. Press **Start** when prompted after loading each plate.
4. Remove the plates from the instrument at the end of the run. Press **Start** when prompted after removing each plate.
5. Press **Stop**.

Extract RNA for qRT-PCR (Automated)

After virus enrichment, the sample can be used for RNA extraction for downstream qRT-PCR using the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit on the KingFisher™ Flex instrument. The procedure can also be performed manually (see Extract RNA for qRT-PCR).

Starting media volume	Resuspension volume
1 mL	200 µL
10 mL	200 µL

Extract RNA for qRT-PCR

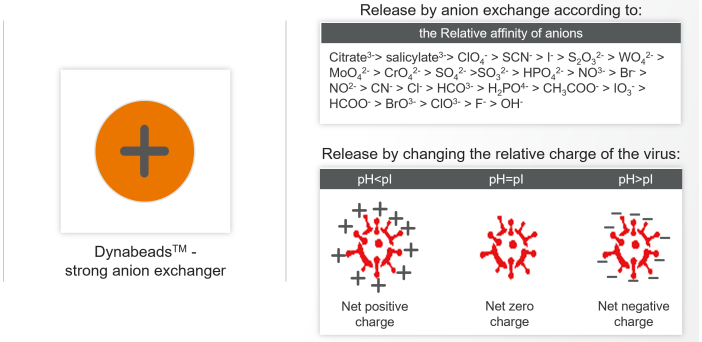
This protocol is based on pre-enrichment of SARS-CoV-2 virus, but can be modified for other viruses.

1. Resuspend the bead-bound virus pellet in 1X PBS with the volumes indicated in the table.
2. Add 250 µL of MagMAX™ Viral/Pathogen Kit Binding Buffer.
3. Transfer the solution to a KingFisher™ 96 deep-well plate (label as sample plate).
4. Add 10 µL of the MagMAX™ Viral/Pathogen magnetic beads to the labelled sample plate.
5. Proceed with the RNA extraction with MagMAX™ Viral/Pathogen Kit following the guidelines used for the KingFisher™ instrument.
6. Elute in a final volume of 10–50 µL.

   Note: Use an elution volume of 10 µL if setting up the qRT-PCR using the TaqPath™ COVID-19 Combo Kit for downstream analysis of viral enrichment samples.
7. Remove the elution plate and place it on ice when the protocol is complete.

   Store the extracted nucleic acid at –20°C for short term storage or –80°C for long term storage.

Principle of isolation and release



Related products

Product	Cat. No.
DynaMag™-2 Magnet	12321D
HulaMixer™ Sample Mixer	15920D
EchoLUTION Viral RNA/DNA Swab Kit Plus	MLS
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit	A42352 A48383
TaqPath™ COVID-19 Combo Kit	A47814
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
SARS/SARS-Cov-2 Coronavirus Nucleocapsid Monoclonal Antibody	MA5-29981

Limited product warranty

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Thermo Fisher Scientific Baltics UAB |
V.A. Graiciuno 8, LT-02241 |
Vilnius, Lithuania

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Revision	Date	Description
B.0	2 March 2023	• Name will changed by removing "optimized for SARS-CoV-2" in the product name • Information updated to inclue several viruses • Some buffer information changed
A.0	7 January 2021	New document for Dynabeads™ Intact Virus Enrichment.