Introducing Dynabeads Speakers



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Education: Ph.D: EMBL Germany/UiO,

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Thermo Fisher Scientific Employee: 15 years

<u>Expertise:</u> EM, cell isolation, exosomes, immunoprecipitation, western, automation, Dynabeads



Berit Marie Reed Global Product Manager, Oslo, Norway

Education: Master in Molecular and cellular biology, University of Oslo (UiO)

Thermo Fisher Scientific Employee >20 years

<u>Expertise:</u> cell isolation, exosomes, immunoprecipitation, automation, Dynabeads



Invitrogen Dynabeads™ Intact Virus **Enrichment** (optimized for SARS-CoV-2)

Ketil W Pedersen **Berit Marie Reed**

> The Dynabeads™ magnetic beads are sold excusively by Veritas Corporation in Japan.



The world leader in serving science

Agenda



Introduction to Dynabeads magnetic beads

Introduction to SARS-CoV-2 and enrichment strategies

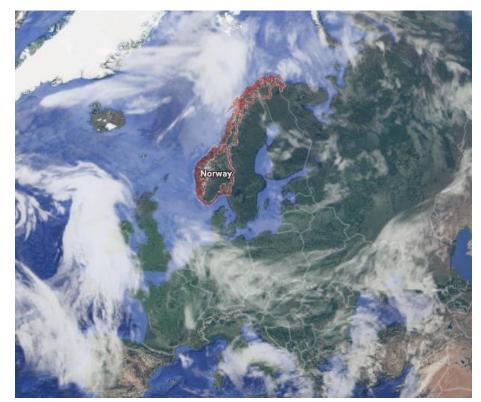
Enrichment of SARS-CoV-2 with Precipitation Reagent

Enrichment of SARS-CoV-2 with Dynabeads

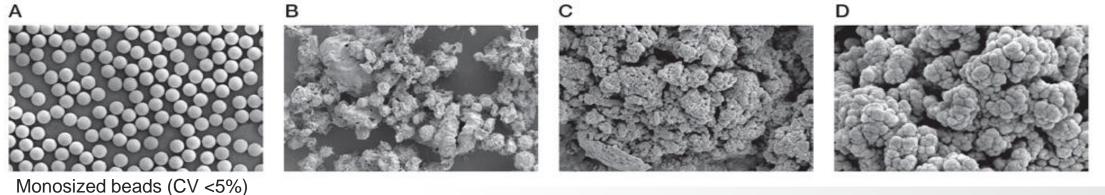
Wastewater testing and summary



What are Dynabeads™ Magnetic Beads?

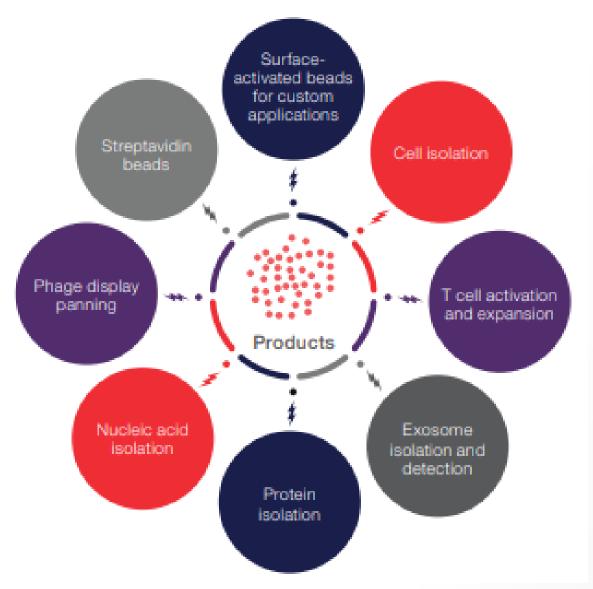


- ✓ Dynabeads products >35 years! The bead pioneers!
- ✓ Only company with truly monosized magnetic beads
- ✓ >80 000 Scientific publications
- ✓ Used in several billions IVD tests annually (OEM)
- ✓ Known for high reproducibility, low non-specific binding and ease of use

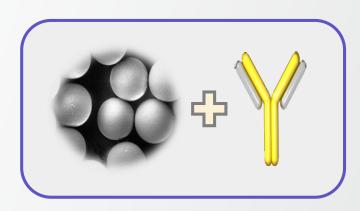


Thermo Fisher SCIENTIFIC

One Technology – Endless Options!

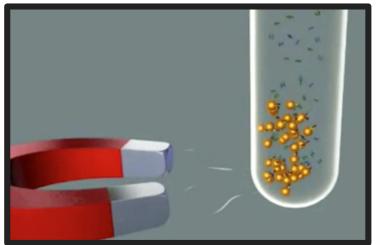


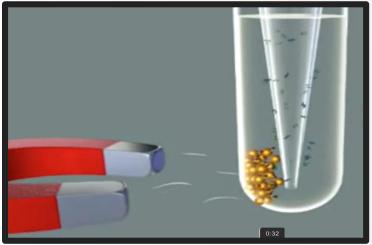
If there is an antibody towards your target, or if it's biotinylated, it can be isolated using Dynabeads products

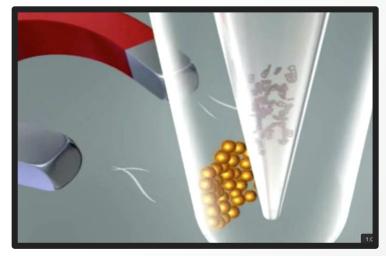


Norway manufacturing site –ISO 13485 certified

How it Works



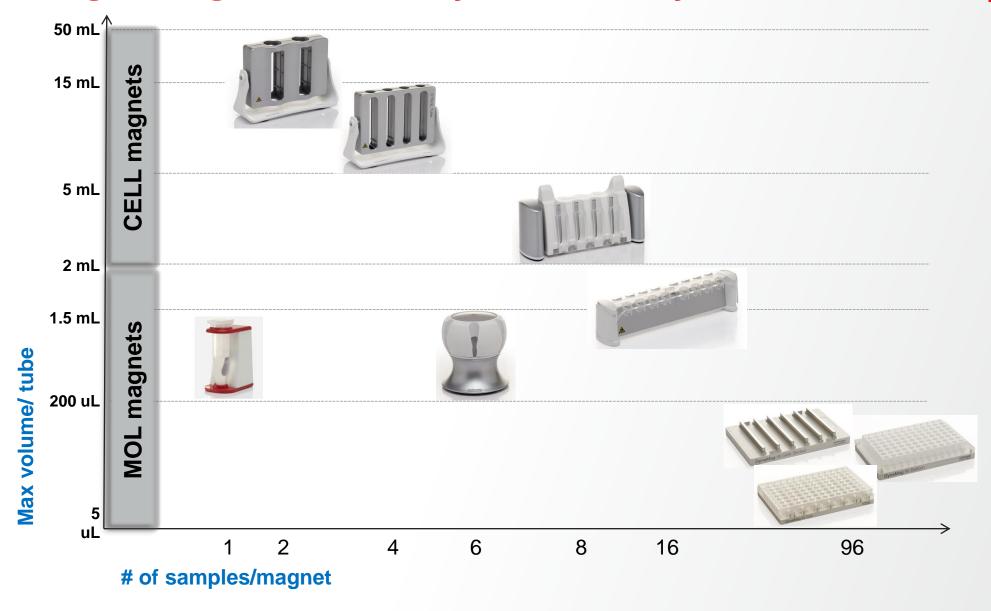




- Dynabeads can be handled
 - Manually (DynaMag™ magnets)
 - Automated (KingFisher™ instruments)



DynaMag™ Magnets – Quality & Flexibility for All Needs



Thermo Fisher SCIENTIFIC

Mixer & Instruments

HulaMixer™ Sample Mixer



- Adjustable—speed range from 1–100 rpm
- Flexible—combine end-over-end rotation, tilting, and vibration (vortex)
- Versatile—two platforms are supplied to match a variety of tubes (0.5 mL—50 mL)
- Can be used in the cold room, at the bench, or in the incubator

KingFisher™ Instruments







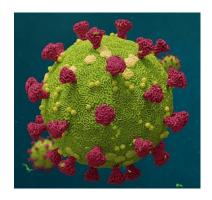


Introduction



Virus enrichment challenges:

- Virus cultures—for sufficient virus vield
- Genome amplification—low virus titers introduce artificial variants or bias in the sequence reads
- Virus surveillance—time-consuming steps and large equipment for enrichment (e.g. ultracentrifugation)



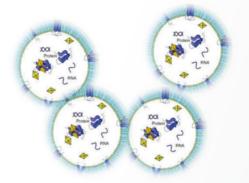
Purpose:

- 1. Purify intact SARS-CoV-2 virus from dilute samples with few steps
- 2. Automate purification of SARS-CoV-2 for high throughput in <30 min

Virus isolation

Description

Solution



- Virus concentration
- Viral characterization
- Viral host interaction
- 1. <u>Precipitation</u> solution for manual virus purification from dilute samples
- 2. Invitrogen[™] <u>Dynabeads</u>[™] magnetic beads for high throughput automated virus purification

Workflow

Manuel and automated enrichment of SARS-CoV-2 virus

Isolation methods **Starting samples** Virus transport media Manual procedure Cell culture media Automated procedure Infectious virus Inactivated virus Virus Like Particles (VLP's)

Applications

- Virus culture
- Wastewater surveillance
- Functional assays
- Serological/Ab
- Vaccine/therapy R&D
- Proteomics
- Genomics

Precipitation reagent and Dynabeads beads enriches all virus types

Current Virus Enrichment Procedures



CMVs

Electronegative membranes

Dengue Virus

Influenza virus

Polyethylen glycol

Tobacco Mosaic Virus

Adenovirus

Coronavirus

Filtration

Bacteriophages

Virus Like Particles

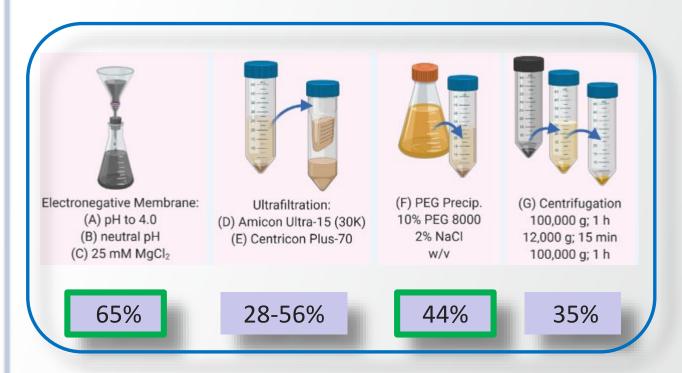
Sucrose gradients

Hepatitus virus

Enveloped virus

Lenti virus

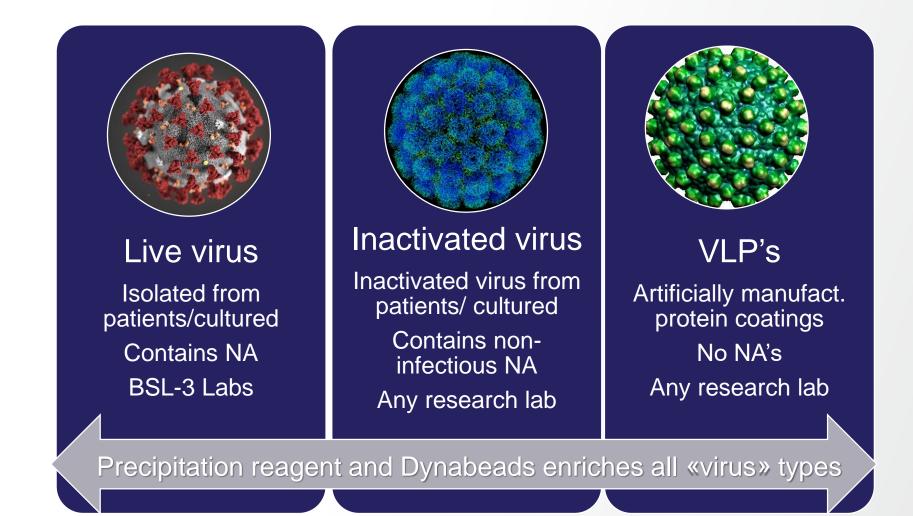
Ultracentrifugation



PEG and charge-based enrichments methods gives best results according to publications

The Lancet May 2020 https://www.microscopemaster.com/Electron-microscopy-images-of-SARS-CoV-2.html

SARS-CoV-2 Research





Precipitation Reagent Benefits and Protocol



Intact Virus Precipitation Reagent (optimized for SARS-CoV-2)

Gentle precipitation method that enriches intact SARS-CoV-2 virus from dilute sample volumes

Benefits

- **Simple-** 3-step virus enrichment
- Fast- Isolate in <3 hours with short hands-on time
- **Flexible -** Dilute virus samples can be enriched from any sample volume range
- **Straight to PCR-** No further NA extraction is needed prior to PCR



Protocol

Add precipitation reagent to the virus sample



Incubate 2 hrs

Centrifuge 12 000 x g

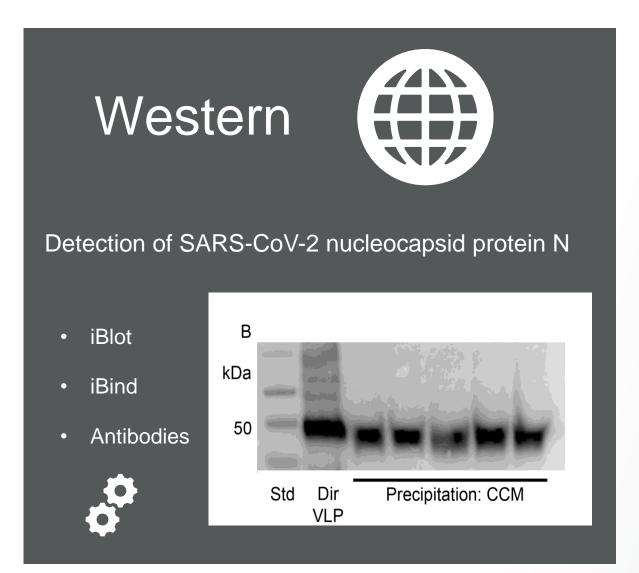


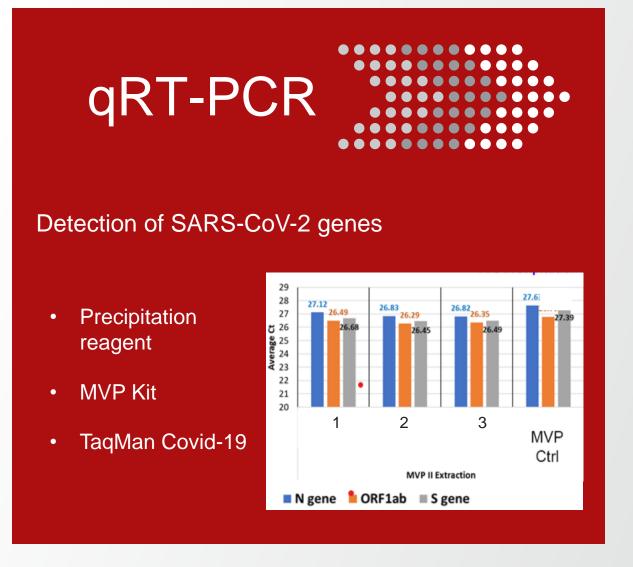
15-30 min

Remove supernatant & collect the virus pellet



Precipitation Reagent is Compatible with Proteomic and **Genomic Workflows**



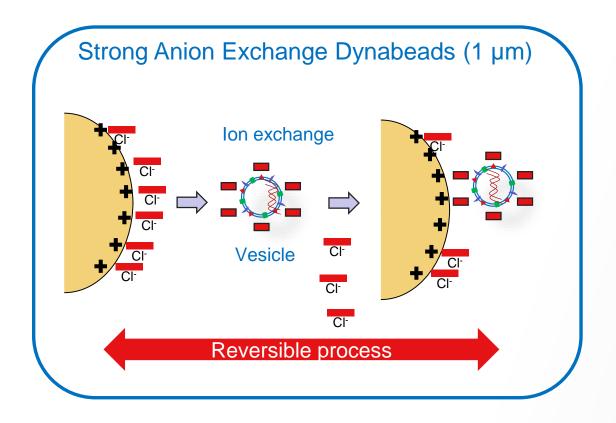




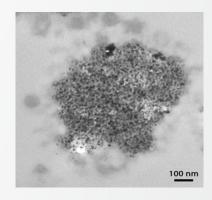


The Dynabeads Isolation Principle

Magnetic bead-based isolation by charge



- Fast isolation kinetics (~10 min)
- Easy to release (~10 min)
- Easy to automate (KingFisher™ instruments)
- Can be used on other negatively charged enveloped viruses, vesicles and proteins



Dynabeads Benefits and Protocol



<u>Dynabeads</u>™ Intact Virus Enrichment (optimized for SARS-CoV-2)

Gentle and fast automated isolation of intact SARS-CoV-2

Benefits

- Fast Intact virus in ~20 minutes
- Simple Just push the button & walk away
- Throughput Enrich up to 96 samples per run
- Functional The intact virus can be used in any functional assay
- Release- Release virus from the beads in 10 min



- SKU #10700D/10701D
- Volume 2 mL/10 mL
- #Rxn's: 100/500

Protocols

1 Automated protocol

Add Dynabeads, virus & buffers to the plates- push "Start"

20 min*

*+10 min w/release

2 Manual protocol

Wash the beads & add to the virus sample

Inc

Incubate 10 min

Wash the beads w/virus



Alternatively, add buffer to release the virus from the beads

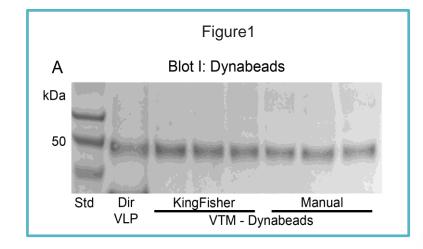


10 min

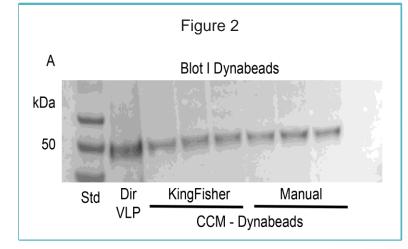
R&D Data for Dynabeads- Western blot

Automated vs. manual isolation of VLP's from CCM and VTM media on KingFisher™ DuoPrime

From VTM





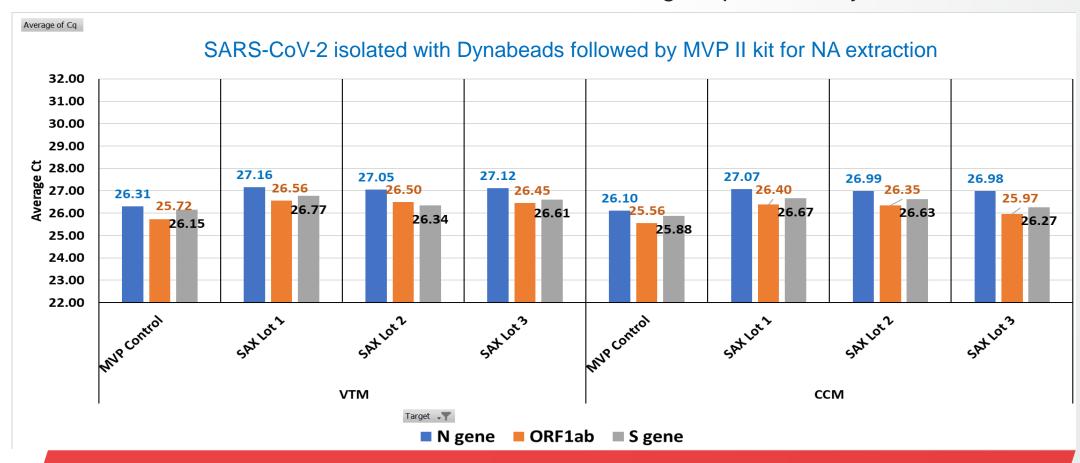


- From VTM- 5 parallel samples
- From CCM- 5 parallel samples
- Protocol time
 - Automated (20 min),
 - Manual (28 min)
- Detection of SARS-CoV-2 nucleocapsid protein N Compared with VLP directly from the vial (Dir VLP)



R&D Data for Dynabeads- qRT-PCR

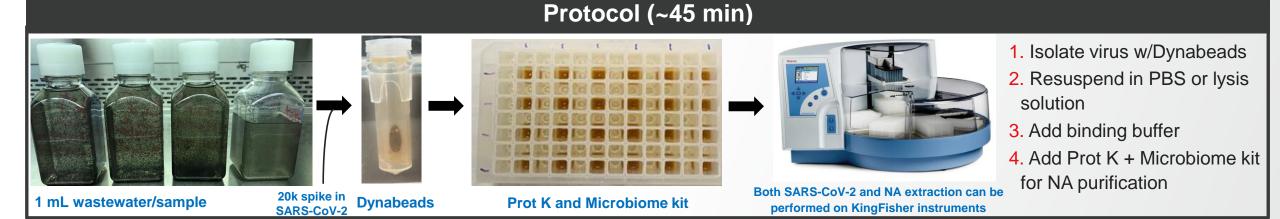
Enrichment of SARS-CoV-2 from VTM and CCM with high reproducibility

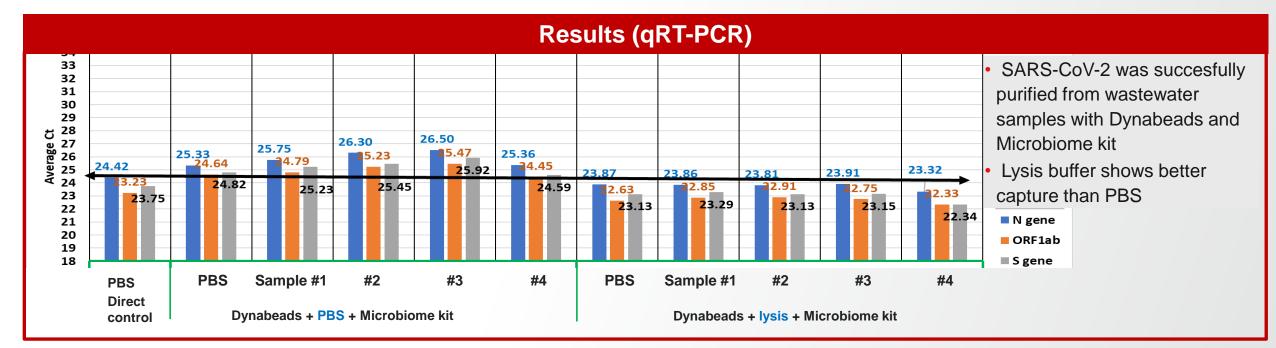


- Very reproducible results between different Dynabeads lots
- Dynabeads isolation of SARS-CoV-2 followed by MVP II extraction matches the sensitivity of MVP control

SARS-CoV-2 Isolated from Wastewater Samples

Dynabeads™ Intact Virus Enrichment (optimized for SARS-CoV-2)





Precipitation Reagent vs. Dynabeads For Enrichment

When to choose what product for SARS-CoV-2!

Intact Virus Precipitation Reagent



Preferred.....

- ...for enrichment from large & dilute samples
- ...for simplicity (3 steps & short hands-on time)
- ...when no automation is needed







Dynabeads™ Intact Virus **Enrichment**



Preferred.....

- ...for high throughput automation
- ...when time is critical (<20 min w/release)
- ...for wastewater samples
- ...for downstream mass spec



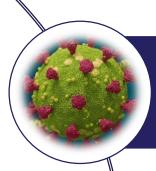






For Research Use only. Not for use in diagnostic procedures.

Summary



Precipitation reagent enriches intact SARS-CoV-2 from dilute samples



Dynabeads enriches intact SARS-CoV-2 in <30 min with virus release



Dynabeads enriches intact SARS-CoV-2 with HTTP on KingFisher instruments



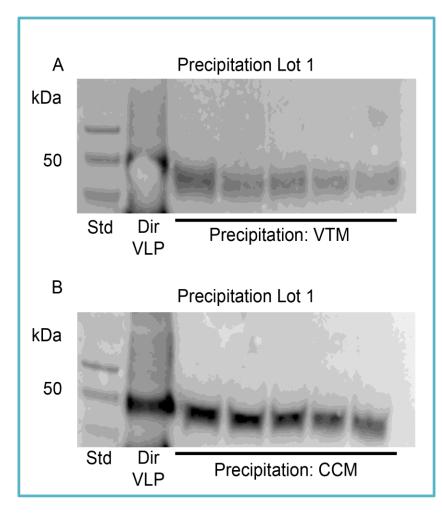
Thank you





R&D Data for Precipitation Reagent- Western blot

Isolation of Virus Like Particles (VLP's) from Cell Culture Media (CCM) and Virus Transport Media (VTM)



- Detection of SARS-CoV-2 nucleocapsid protein N (50 kDa)
- Compared with VLP directly from the vial (Dir VLP).
- 2 hours 40 min isolation time
- Very reproducible between different lots and parallels

