

Introducing Dynabeads Speakers



Ketil Winther Pedersen, Ph.D
Manager R&D, Oslo, Norway

Education: Ph.D: EMBL Germany/UiO,
post doc I: LUMC (Leiden, the Netherlands),
post doc II: National Hospital Norway.

Thermo Fisher Scientific Employee: 15 years

Expertise: 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

Expertise: cell isolation, exosomes,
immunoprecipitation, automation, Dynabeads

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

Ketil W Pedersen
Berit Marie Reed

 The world leader in serving science

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

Agenda

1 Introduction to Dynabeads magnetic beads

2 Introduction to SARS-CoV-2 and enrichment strategies

3 Enrichment of SARS-CoV-2 with Precipitation Reagent

4 Enrichment of SARS-CoV-2 with Dynabeads

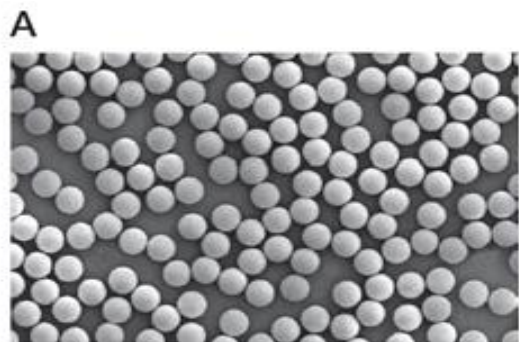
5 Wastewater testing and summary

“There is
no shortcut
to achievement.”

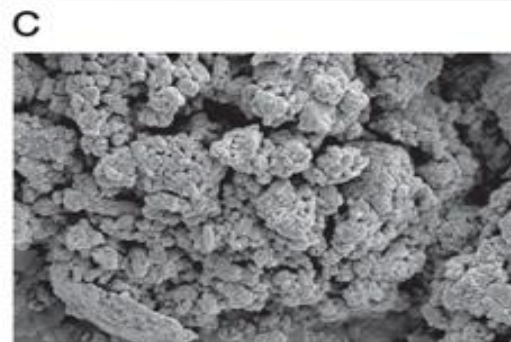
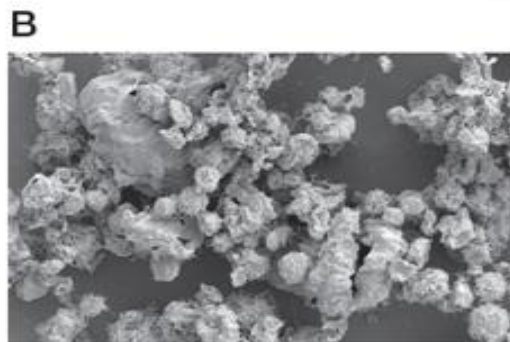
What are Dynabeads™ Magnetic Beads?



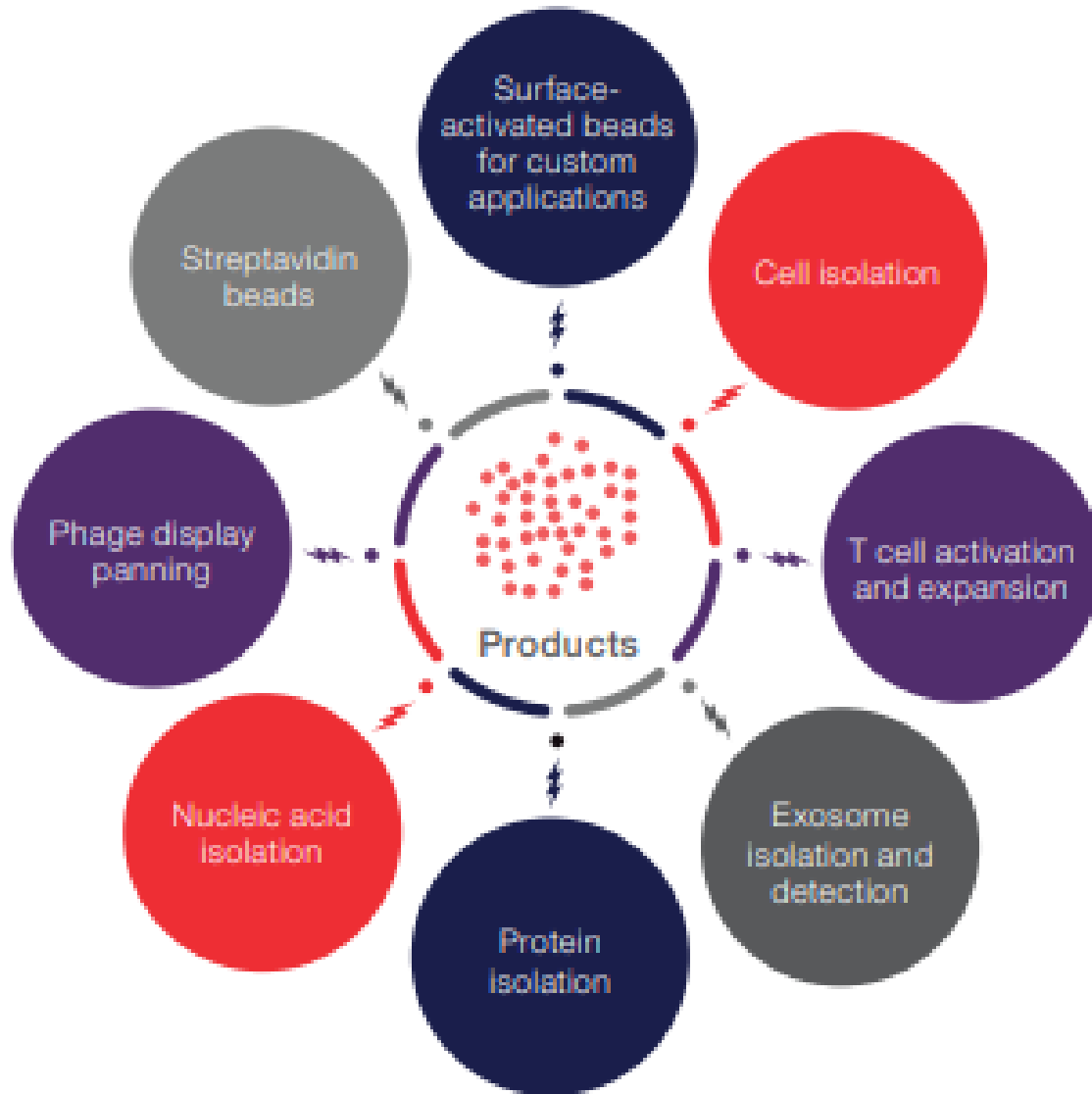
- ✓ Dynabeads products >35 years! The bead pioneers!
- ✓ Only company with truly monosized magnetic beads
- ✓ >80 000 Scientific publications
- ✓ Used in several billions IVD tests annually (OEM)
- ✓ Known for high reproducibility, low non-specific binding and ease of use



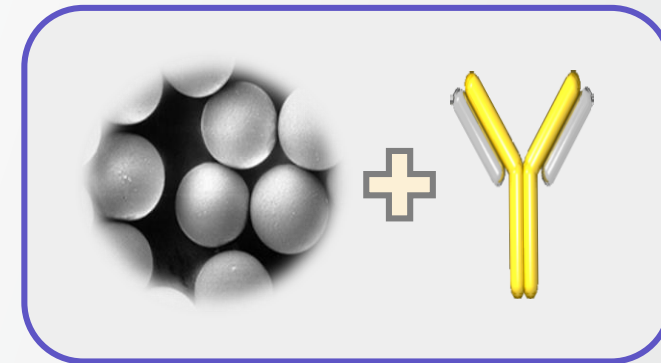
Monosized beads (CV <5%)



One Technology – Endless Options!

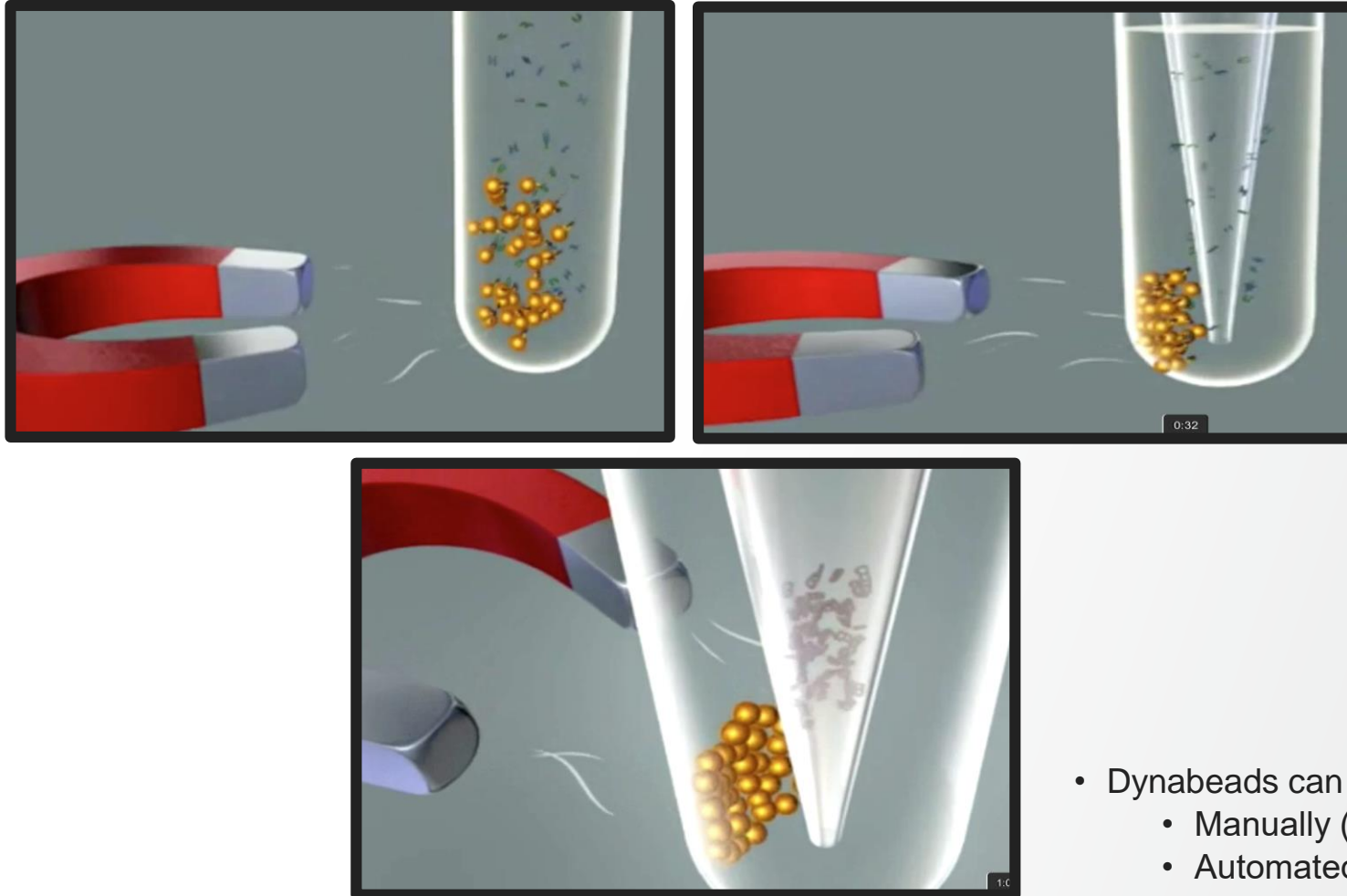


If there is an antibody towards your target, or if it's biotinylated, it can be isolated using Dynabeads products



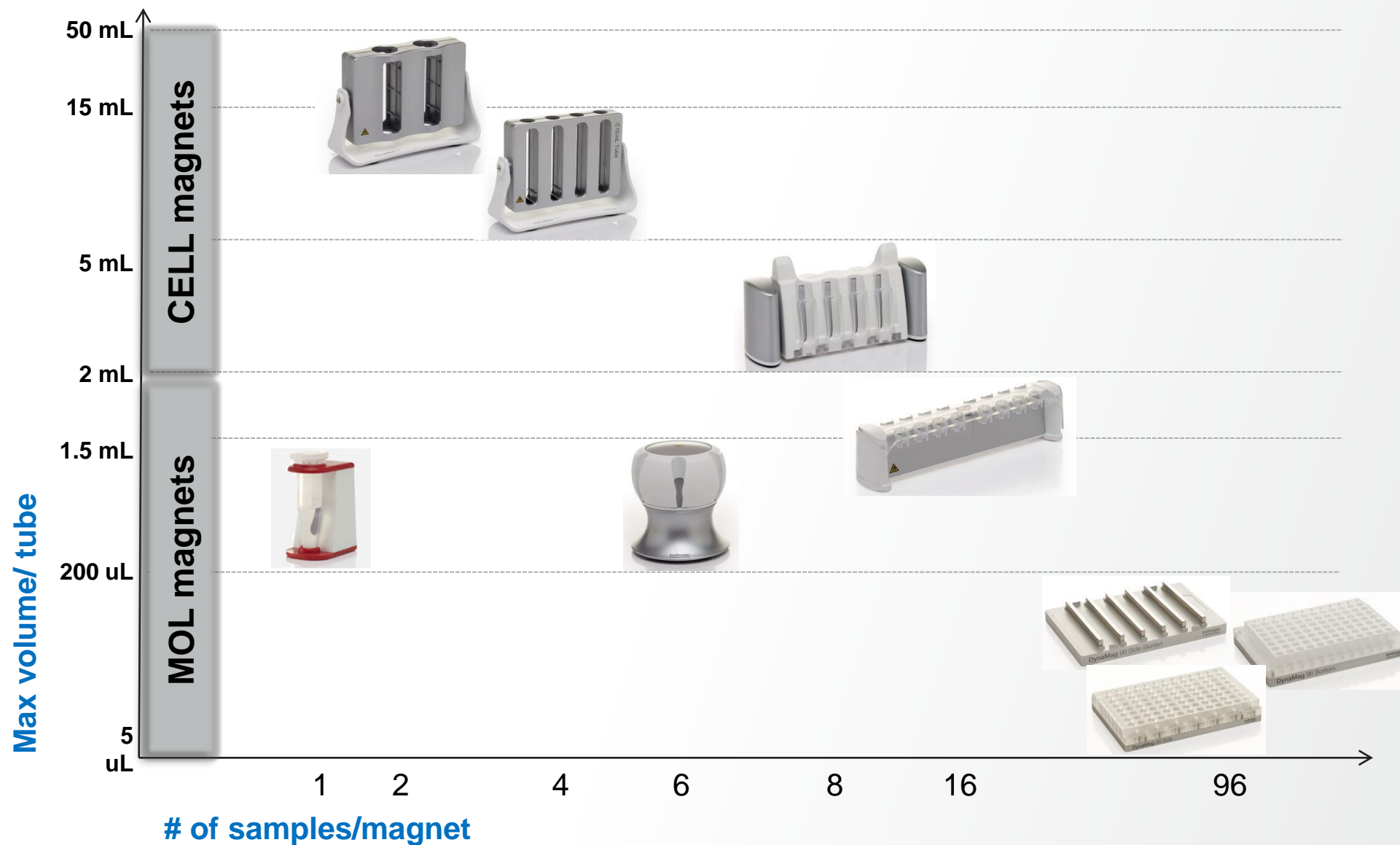
Norway manufacturing site –ISO 13485 certified

How it Works



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

DynaMag™ Magnets – Quality & Flexibility for All Needs



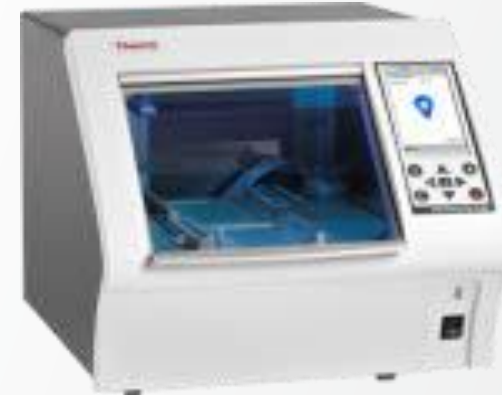
Mixer & Instruments

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



Duo Prime 6-12 rxn/run



Flex 24/96 rxn/run



Apex 24/96 rxn/run

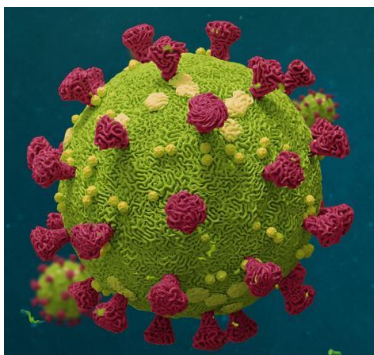


Introduction to SARS-CoV-2

Introduction

Virus enrichment challenges:

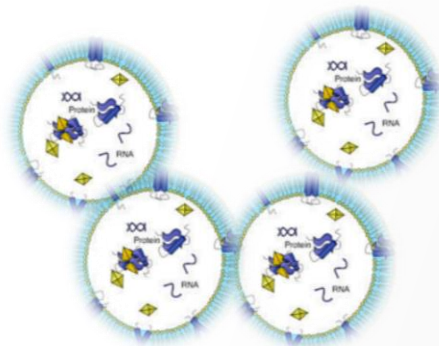
- **Virus cultures**—for sufficient virus yield
- **Genome amplification**—low virus titers introduce artificial variants or bias in the sequence reads
- **Virus surveillance**—time-consuming steps and large equipment for enrichment (e.g. ultracentrifugation)



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

- Virus concentration
- Viral characterization
- Viral host interaction

Solution

1. Precipitation solution for manual virus purification from dilute samples
2. Invitrogen™ Dynabeads™ magnetic beads for high throughput automated virus purification

Workflow

Manuel and automated enrichment of SARS-CoV-2 virus



Precipitation reagent and Dynabeads beads enriches all virus types

Current Virus Enrichment Procedures

CMVs

Electronegative membranes

Dengue Virus

Influenza virus

Tobacco Mosaic Virus

Polyethylen glycol

Adenovirus

Coronavirus

Filtration

Bacteriophages

Virus Like Particles

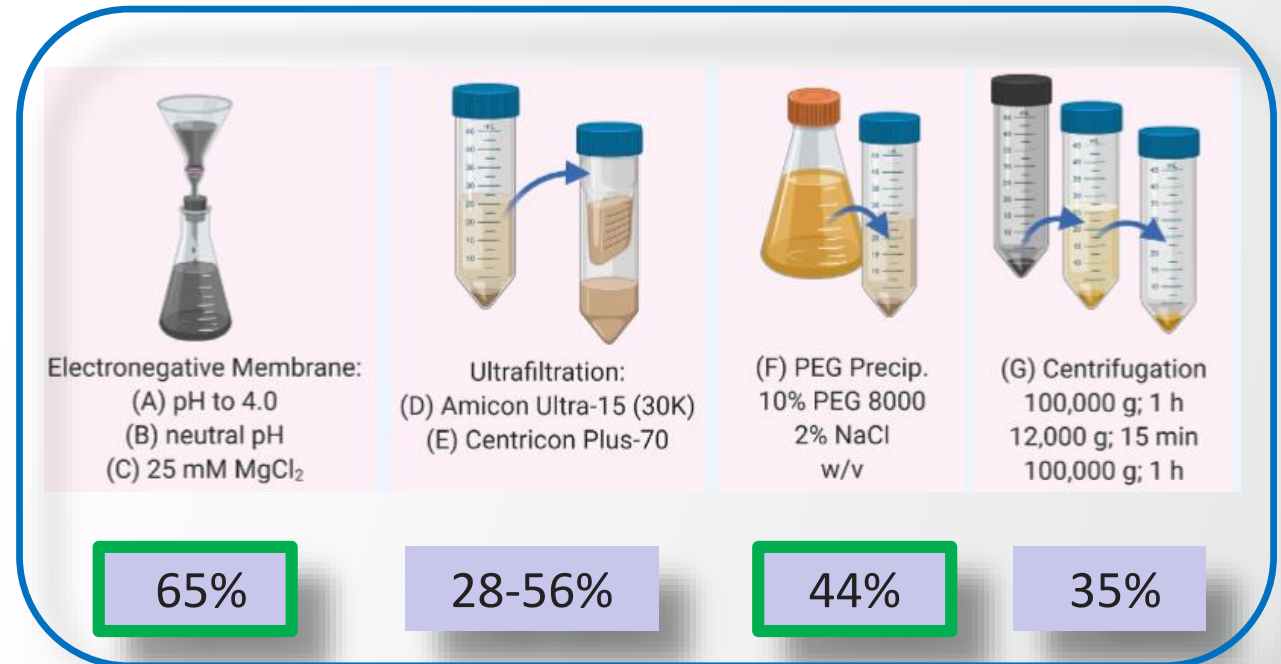
Sucrose gradients

Hepatitis virus

Enveloped virus

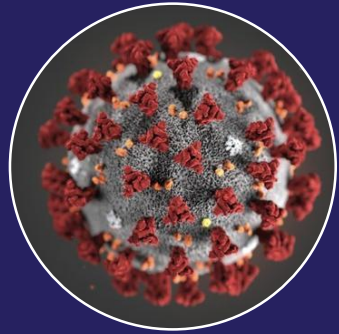
Lenti virus

Ultracentrifugation



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

SARS-CoV-2 Research

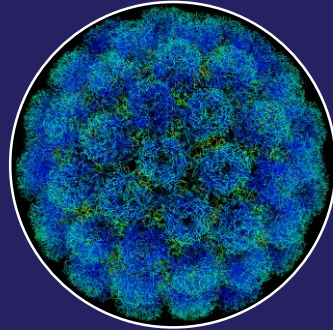


Live virus

Isolated from patients/cultured

Contains NA

BSL-3 Labs

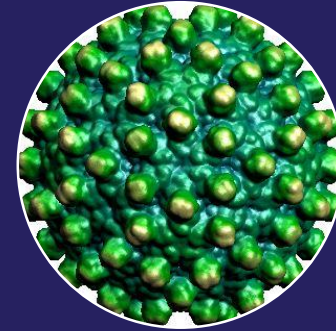


Inactivated virus

Inactivated virus from patients/ cultured

Contains non-infectious NA

Any research lab



VLP's

Artificially manufact. protein coatings

No NA's

Any research lab

Precipitation reagent and Dynabeads enriches all «virus» types



Precipitation Reagent

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



- SKU #10720D
- Volume - 50 mL
- #Rxn's: 100*

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

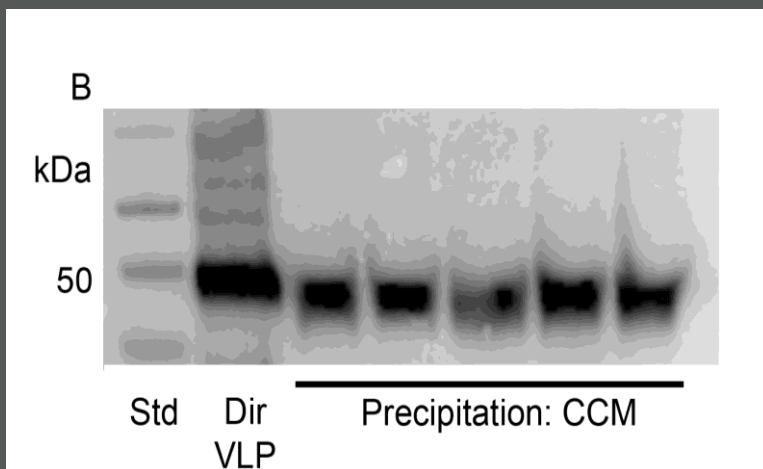
Precipitation Reagent is Compatible with Proteomic and Genomic Workflows

Western

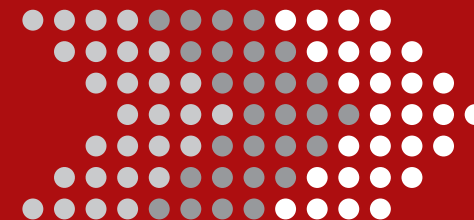


Detection of SARS-CoV-2 nucleocapsid protein N

- iBlot
- iBind
- Antibodies

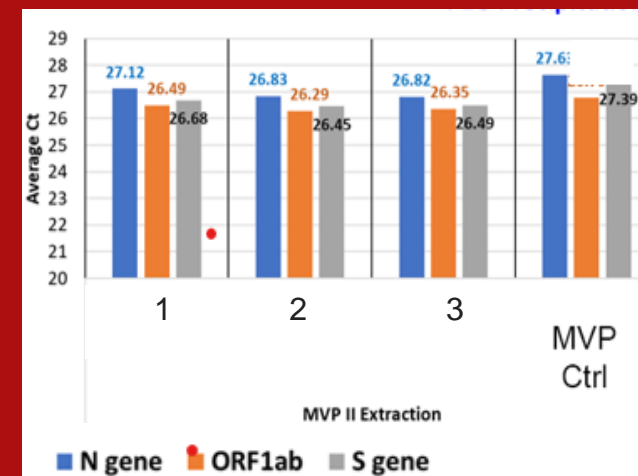


qRT-PCR



Detection of SARS-CoV-2 genes

- Precipitation reagent
- MVP Kit
- TaqMan Covid-19

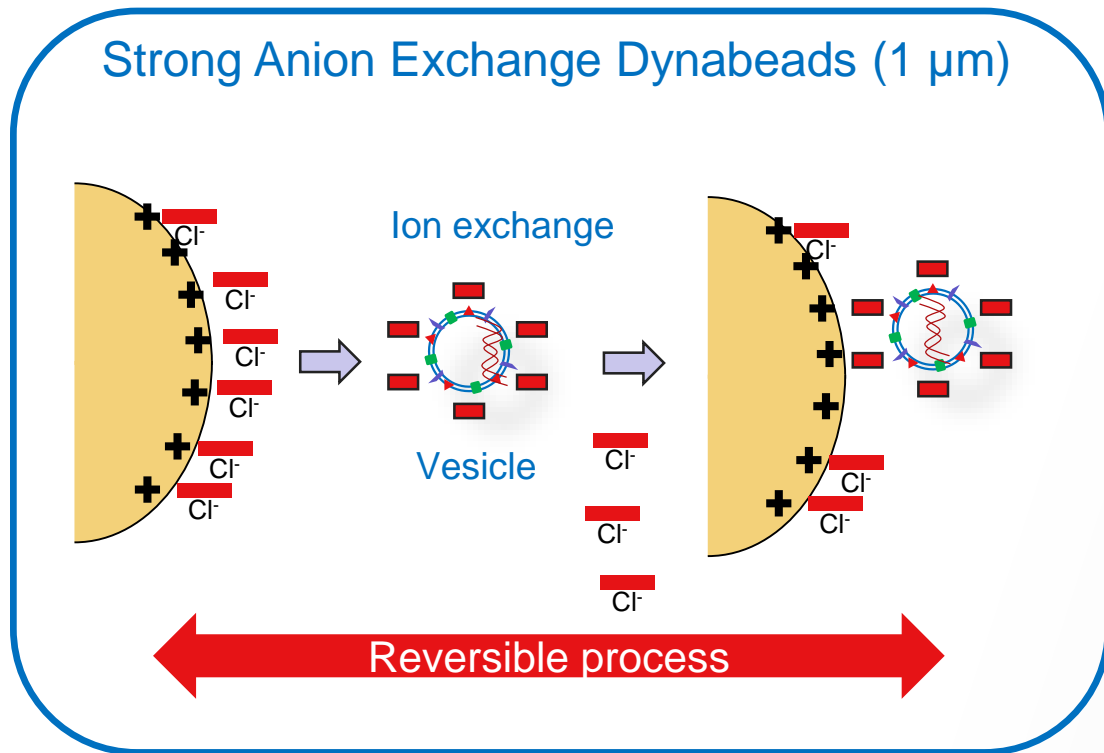


A close-up photograph of a woman with dark hair and eyes, looking intently at a large, stylized virus particle. The virus is green with numerous red, spike-like protrusions. The background is a soft, out-of-focus blue and white.

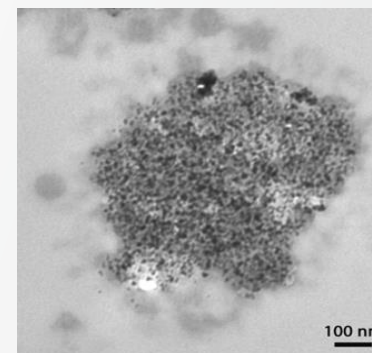
Dynabeads™ for automation

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

Dynabeads™ 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

Incubate 10 min

Wash the beads w/virus

2 min

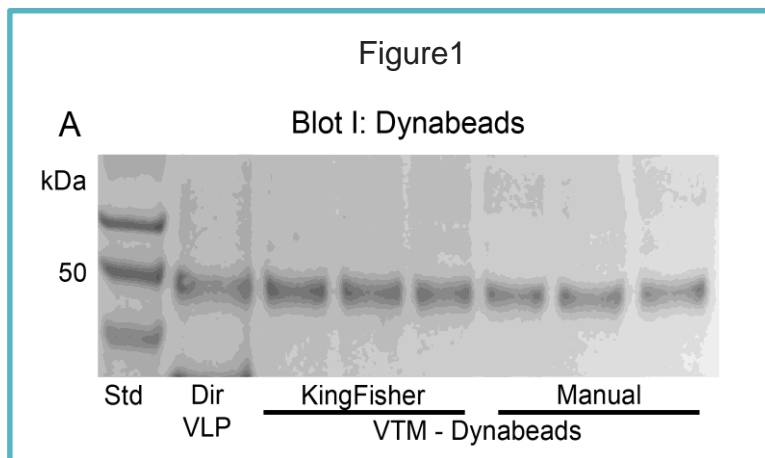
Alternatively, add buffer to release the virus from the beads

10 min

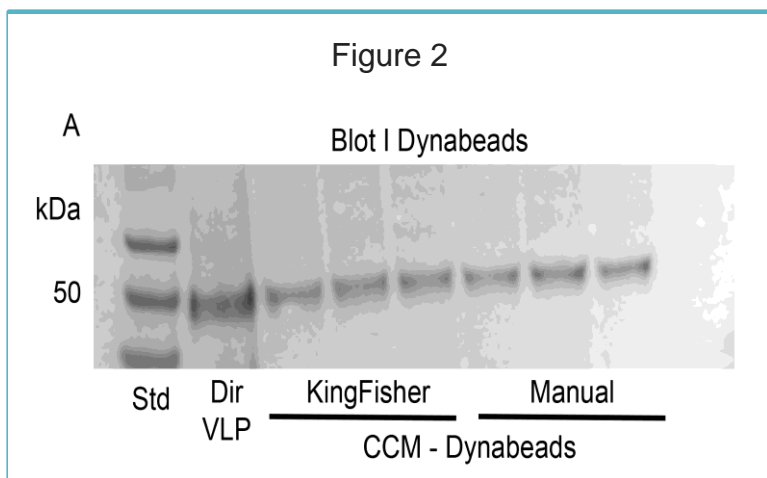
R&D Data for Dynabeads- Western blot

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

From VTM



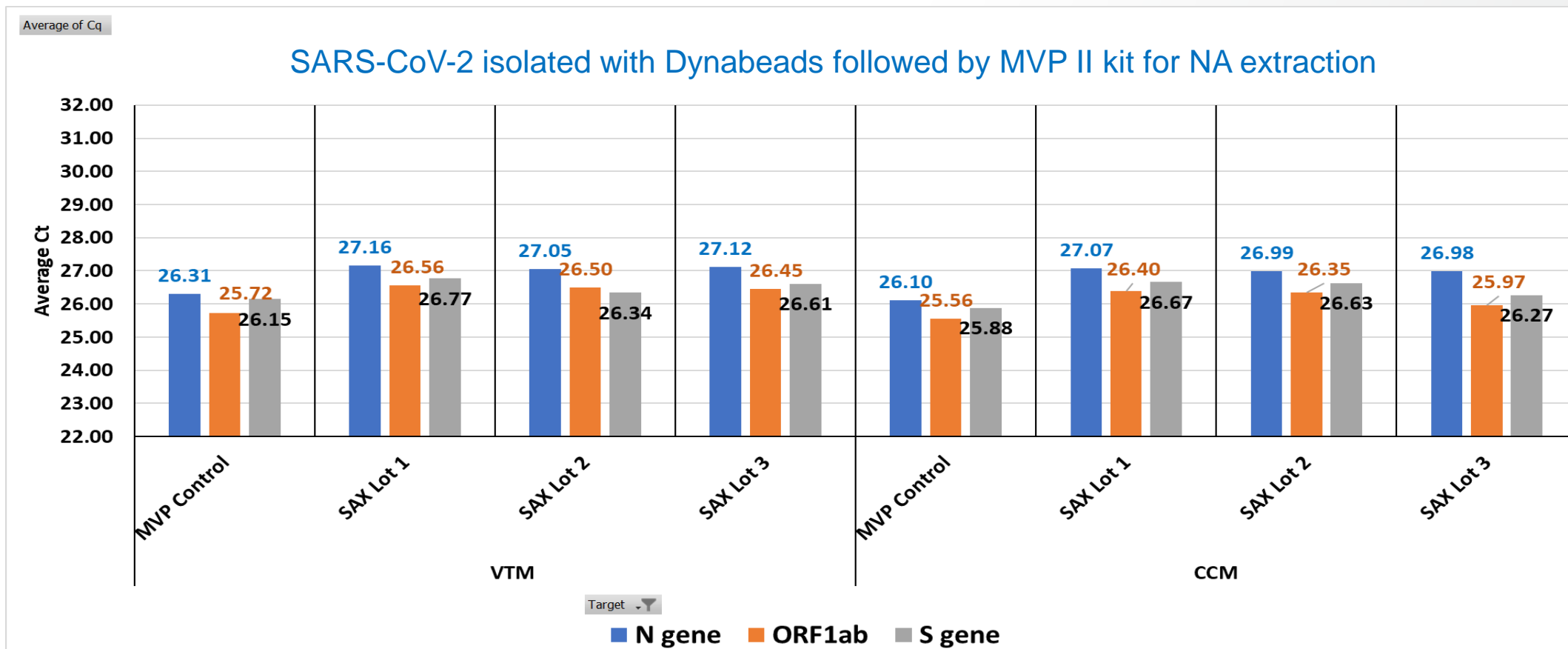
From CCM



- 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

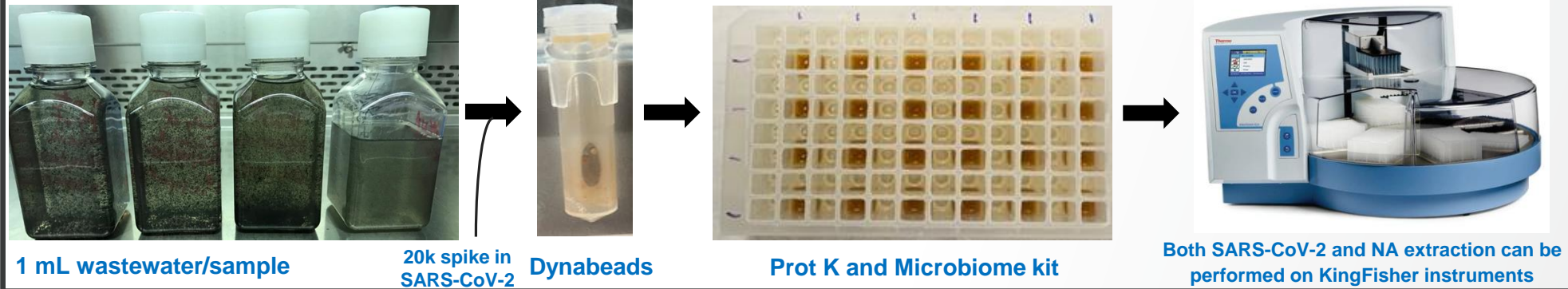


- Very reproducible results between different Dynabeads lots
- Dynabeads isolation of SARS-CoV-2 followed by MVP II extraction matches the sensitivity of MVP control

SARS-CoV-2 Isolated from Wastewater Samples

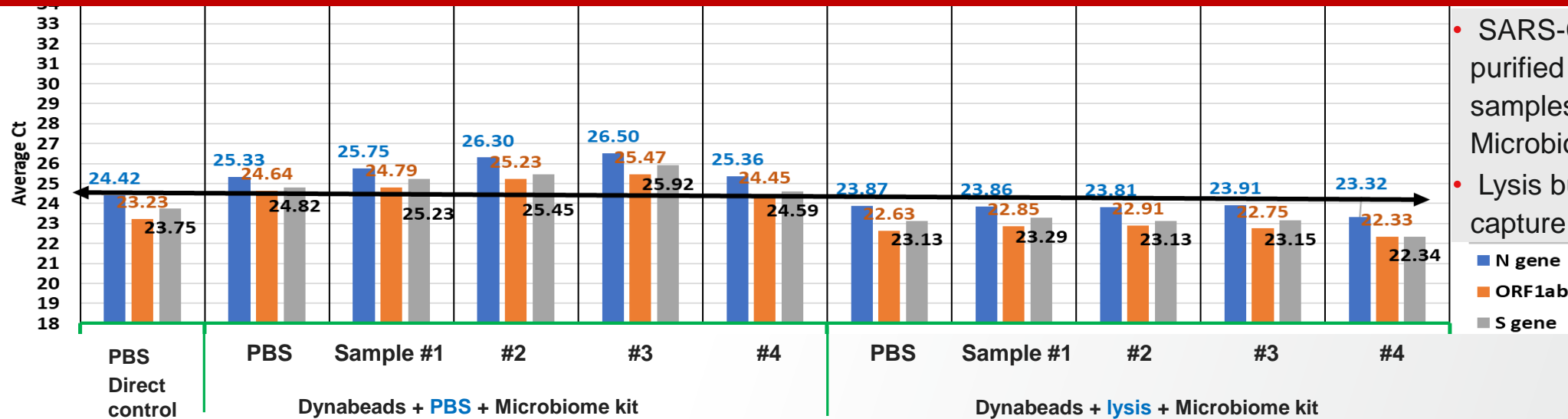
Dynabeads™ Intact Virus Enrichment (optimized for SARS-CoV-2)

Protocol (~45 min)



1. Isolate virus w/Dynabeads
2. Resuspend in PBS or lysis solution
3. Add binding buffer
4. Add Prot K + Microbiome kit for NA purification

Results (qRT-PCR)



- SARS-CoV-2 was successfully purified from wastewater samples with Dynabeads and Microbiome kit
- Lysis buffer shows better capture than PBS

Precipitation Reagent vs. Dynabeads For Enrichment

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

Intact Virus Precipitation Reagent

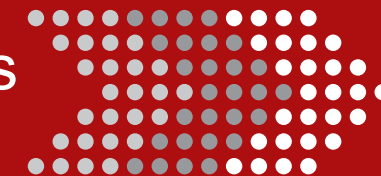


Preferred.....

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



Dynabeads™ Intact Virus Enrichment



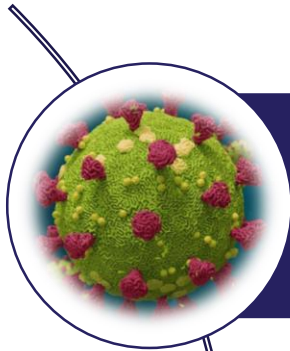
Preferred.....

- ...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

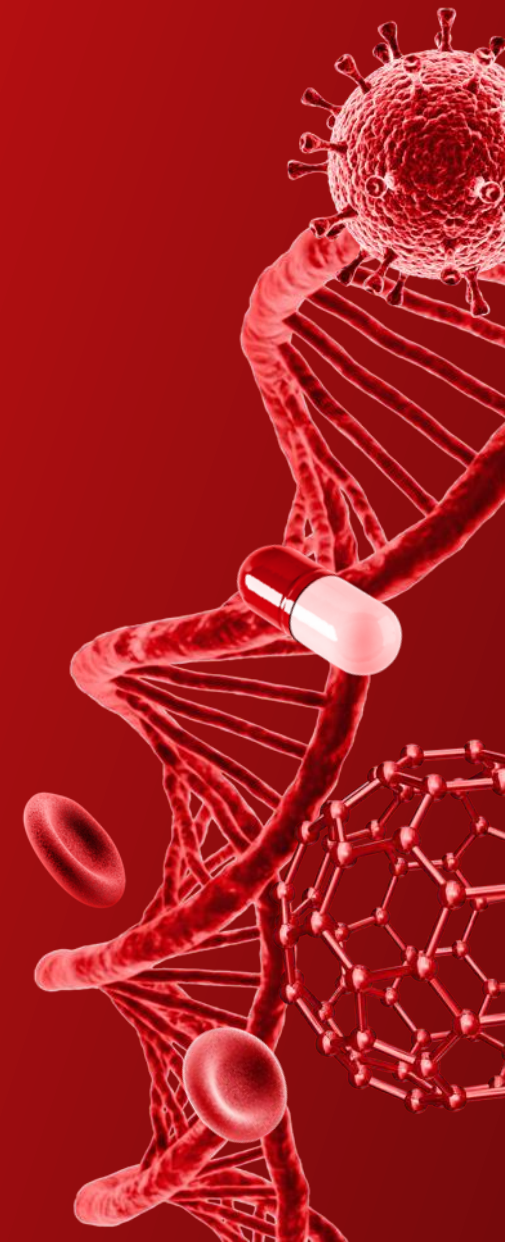


Dynabeads enriches intact SARS-CoV-2 in <30 min with virus release



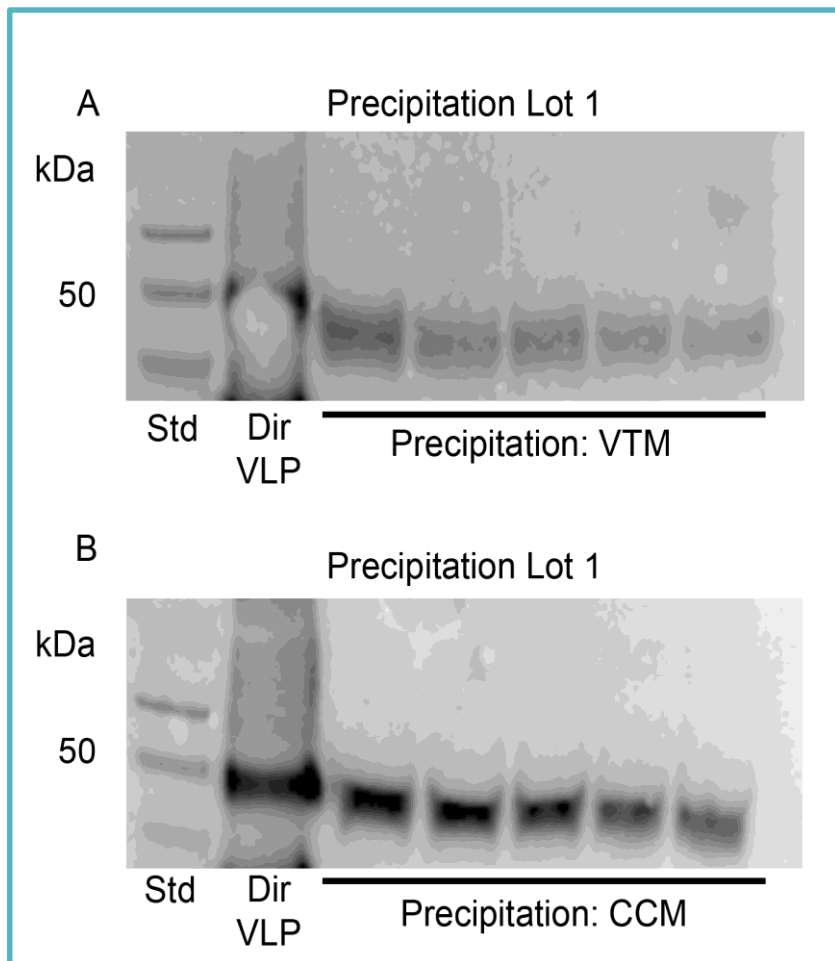
Dynabeads enriches intact SARS-CoV-2 with HTTP on KingFisher instruments

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

