NEB UK expressions

JAN 2023

Free! 2023 NEB Wall Calendar

NEW PRODUCTS

Induro™ Reverse Transcriptase

Ph.D.™ Phage Display Peptide Library Kits v2

SPECIAL OFFER

Get a free pack of NEBees™ honey with purchases of selected Monarch Kits!

APPLICATION NOTE

Multiplex real-time PCR detection of monkeypox virus using Luna® qPCR Reagents

NEW ENGLAND BioLabs®

CONTENTS

Understanding the Monarch Butterfly

Available at
neb.com/podcasts/nebpodcast

NEW ENGLAND BioLabs®

be INSPIRED
drive DISCOVERY
stay GENUINE

New England Biolabs (UK) Ltd
Tel: 0800 318486 | Email: info.uk@neb.com | www.neb.uk.com

Cover photo: By AllaSaa / Shutterstock.com

SPECIAL OFFER

Get a free pack of NEBees honey with purchases of selected Monarch Kits!

+

From 1st January until 31st March 2023, if you purchase a large pack of selected Monarch Kits you will receive a free pack of honey made by our NEBees! Each pack contains 3 mini jars of delicious NEBees honey from the first harvest of the hives we have sponsored near NEB UK in Hertfordshire.

Qualifying Products

Turn to pages 4 and 5 to learn more about these Monarch Kits.

PRODUCT	NEB #	PACK SIZE
Monarch Plasmid Miniprep Kit	T1010L	250 Preps
Monarch DNA Gel Extraction Kit	T1020L	250 Preps
Monarch PCR & DNA Cleanup Kit (5 µg)	T1030L	250 Preps

For UK pricing please visit www.neb.uk.com

**TERMS & CONDITIONS: From 1st January 2023 until 31st March 2023, for every purchase* of #T1010L, T1020L and T1030L, the purchaser (end-user) will receive a free pack of 3 mini jars of NEBees™ Honey. Valid only for purchases from New England Biolabs (UK) Ltd in the UK from 1/1/23 - 31/3/23. Purchasers from Brennan & Co in Ireland and Northern Ireland will receive an equivalent pack of honey from hives sponsored locally by Brennan & Co. No cash or cash equivalent. No substitution. Valid on new orders. May not be applied to existing, pending or prior orders. Cannot be combined with any other promotions. Void if copied or transferred and where prohibited by law. See www.neb.uk.com/offers for full details. *A limit of 10 packs per purchaser over the period of the offer applies. For pricing information in Republic of Ireland and Northern Ireland please contact Brennan & Co, tel: +353 1 2952501, email: info@brennanco.ie*

Meet our NEBees™

While passion for science helps us to drive discovery, at New England Biolabs we continue to be guided by our responsibility to each other and our community, both locally and globally.

With that in mind, in spring of last year, New England Biolabs UK, in partnership with The Honeybee Man Ltd sponsored the setting up of two new honeybee colonies in an apiary in Weston, Hertfordshire. Along with this sustainable beekeeping company, we will help to drive an increase in the UK honeybee population.

www.neb.uk.com/NEBees

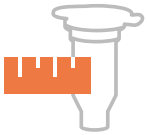
Monarch DNA & RNA Purification Kits – Designed with sustainability in mind

At NEB, we continuously strive to promote ecologically sound practices and environmental sustainability in order to protect our natural resources, both locally and globally. For over 5 years, we have been designing our Monarch DNA & RNA purification kits and products with sustainability in mind by purposefully reducing plastics and packaging without sacrificing performance and quality.

Reduced lab waste

Significantly less plastic as compared to leading supplier

Monarch kits still deliver high yields, purity and performance



Thinner-walled columns

Reduction in total plastic without affecting performance

Buffer bottles

Carefully designed to minimize plastic usage

Flexible purchasing options

Buffers and columns sold separately

Purchase only what you need and avoid wasted materials

Same performance, design and formulations

Standalone products are the same components that are included in complete kits

No excessive packaging

Sturdy, reusable boxes at just the right size

Carefully designed to eliminate empty space, versatile Monarch boxes can be reused anywhere

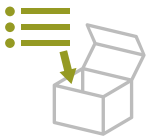
Concise protocol cards replace printed manuals

Both cards and manuals are available online as PDFs

Sustainable & recyclable packaging

Sourced for recyclability

All components are purposefully sourced for recyclability



Instructions for recycling kit components

Can be found on product packing or online

Recycled paper

Used to make the kit boxes, inserts and paper materials

Eco-friendly printing

Printing of boxes and packaging powered by green sustainable sources such as wind

We hope that others in the scientific community join us in our efforts to make nucleic acid purification greener, one step at a time.

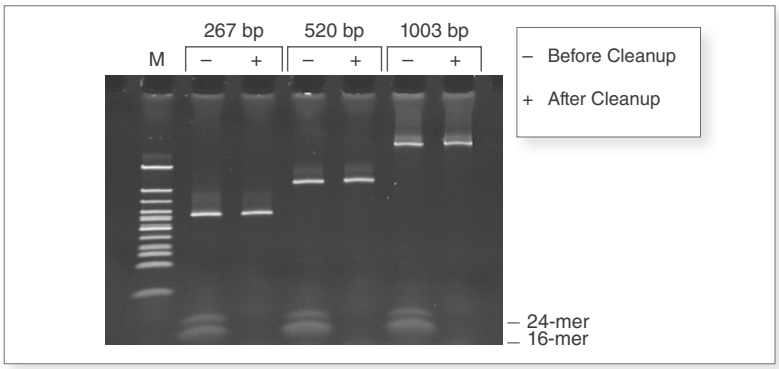
To learn more and request your Monarch sample, visit **NEBMonarch.com**.

Monarch Kits for your DNA Cleanup and Gel Extraction

Monarch PCR & DNA Cleanup Kit (5 µg)

The Monarch PCR & DNA Cleanup Kit (5 µg) can be used to purify DNA from a variety of enzymatic reactions, such as PCR, restriction digestion, ligation and reverse transcription. The DNA Wash Buffer provided ensures enzymes, short primers (≤ 25 nt), detergents and other low-molecular weight reaction components (e.g., nucleotides, DMSO, betaine) are removed. A simple protocol modification also enables purification of small DNA and oligonucleotides.

Monarch PCR & DNA Cleanup Kit (5 µg) removes low molecular weight primers from dsDNA samples

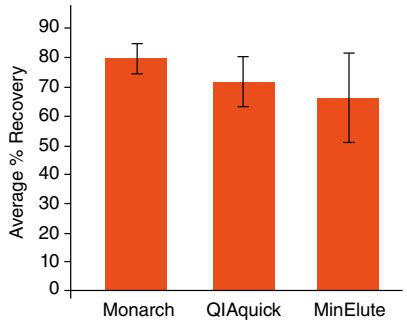


Three independent amplicons (267 bp, 520 bp, 1003 bp) were spiked with two oligonucleotides (16-mer, 24-mer) to a final concentration of 1 µM. Half of each mix was purified with the Monarch PCR & DNA Cleanup Kit (5 µg) following the included protocol. Equivalent fractions of the original mixture and the eluted material were resolved on a 20% TBE acrylamide gel at 100V for one hour and stained with SYBR Green II.

Monarch DNA Gel Extraction Kit

The Monarch DNA Gel Extraction Kit can be used to quickly purify DNA from agarose gels. Unlike other kits, there is no need to add isopropanol to the melted agarose prior to loading on the column, saving you a step. Enjoy high yields and minimal hands on time.

DNA purified from the Monarch DNA Gel Extraction Kit is recovered with similar efficiency and purity as the leading supplier, but is more highly concentrated, facilitating its use in downstream applications



	Monarch	QIAquick®	MinElute®
$A_{260/280}$	1.9+/-0.08	1.9+/-0.08	1.9+/-0.04
Yield (µg)	0.80+/-0.05	0.72+/-0.09	0.66+/-0.13
Conc. (ng/µl)	144.9+/-9.6	25.7+/-3.1	73.8+/-14.2

One microgram aliquots of a 3 kb fragment were resolved on a 1% w/v agarose gel, excised, and processed with different kits using manufacturer-specified minimum elution volumes. Values reported are the concentration and purity data determined by Nanodrop™ readings, as well as recovery calculations based on the eluted DNA concentration and recovered volume.

ADVANTAGES

- Elute in as little as 6 µl
- Prevent buffer retention and salt carryover with optimized column design
- Purify oligos and other small DNA fragments with simple protocol modification
- Save time with fast, user-friendly protocols
- Designed with sustainability in mind
- With protocol modification, DNA ≥ 15 bp (dsDNA) or ≥ 18 nt (ssDNA) can be purified with NEB #T1030

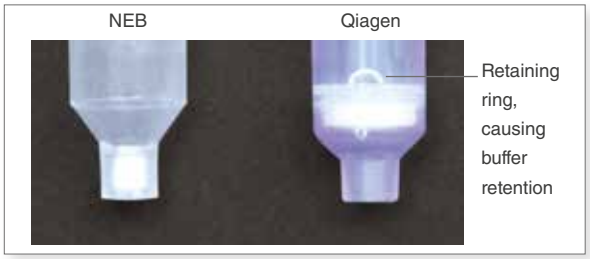
SPECIFICATIONS

- **Binding Capacity:** up to 5 µg
- **DNA Size Range:** ~50 bp to 25 kb
  With protocol modification, oligos ≥ 15 bp (dsDNA) or ≥ 18 nt (ssDNA) can be purified with NEB #T1030
- **Typical Recovery:**
  DNA (50 bp to 10 kb): 70–90%
  DNA (11–25 kb): 50–70%
  ssDNA ≥ 18 nt and dsDNA ≥ 15 bp: 70–85% (NEB #T1030 only)
- **Elution Volume:** ≥ 6 µl
- **Purity:** $A_{260/280}$ ≥ 1.8
- **Protocol Time:**
  Gel Extraction: 10 min of spin and incubation time
  PCR & DNA Cleanup: 5 min of spin and incubation time
- **Compatible Downstream Applications:** ligation, restriction digestion, labeling and other enzymatic manipulations, library construction and DNA sequencing

TIPS FOR SUCCESSFUL GEL EXTRACTIONS

Visit https://international.neb.com/tools-and-resources/usage-guidelines/six-tips-for-a-perfect-gel-extraction to find out more.

Optimized Monarch column design

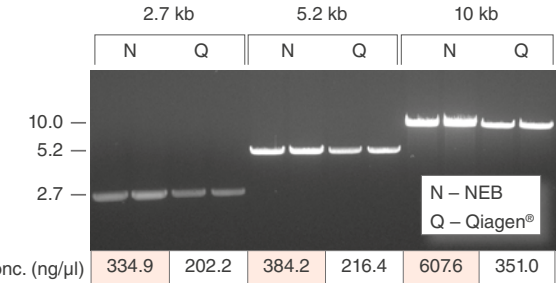


Many purification columns are built with a retaining ring to hold the membrane in place, but this can trap buffer. Monarch columns' silica matrix is held in place without the use of a retaining ring, eliminating buffer retention and ensuring worry-free purification.

Monarch Plasmid Miniprep Kit

The Monarch Plasmid Miniprep Kit is a rapid and reliable method for the purification of high quality plasmid DNA. This method employs standard cell resuspension, alkaline lysis, and neutralization steps, with the additional benefit of color indicators at certain steps to easily monitor completion. Unique wash buffers ensure salts, proteins, RNA and other cellular components are removed, allowing low-volume elution of concentrated, highly pure DNA. Protocols are fast and user-friendly. Elution in as little as 30 µl provides concentrated DNA for use in downstream applications, such as restriction digests, DNA sequencing, PCR and other enzymatic manipulations.

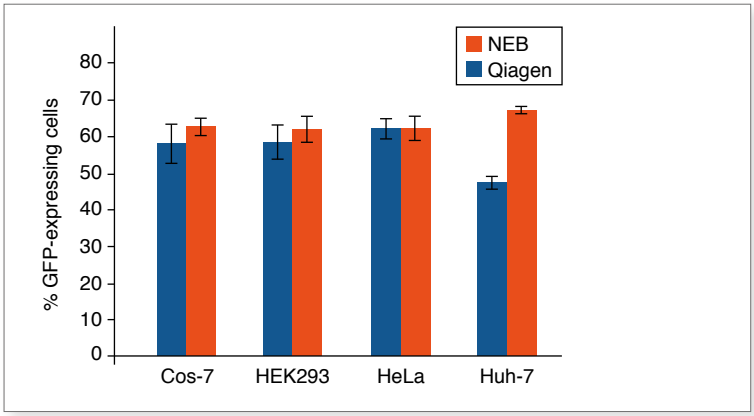
Monarch Plasmid Miniprep Kits consistently produce more concentrated plasmid DNA with equivalent yield, purity and functionality as compared to the leading supplier



	2.7 kb		5.2 kb		10 kb	
	N	Q	N	Q	N	Q
Conc. (ng/µl)	334.9	202.2	384.2	216.4	607.6	351.0
Total yield (µg)	10.0	10.1	11.5	10.8	18.2	17.6
$A_{260/280}$	1.9	1.9	1.9	1.9	1.9	1.9
$A_{260/230}$	2.4	2.3	2.4	2.4	2.3	2.4

Preps were performed according to recommended protocols using 1.5 ml aliquots of the same overnight culture. One microliter of each prep was digested with HindIII-HF® (NEB #R3104) to linearize the vector and the digests were resolved on a 1% w/v agarose gel.

Plasmid DNA purified using the Monarch Plasmid Miniprep Kit produces transfection efficiencies equivalent to or better than plasmid DNA purified using the Qiagen QIAprep® Spin Miniprep Kit



Plasmid DNA encoding constitutively expressed GFP (pEGFP-C2) was prepared using either Monarch Plasmid Miniprep Kit or Qiagen QIAprep Spin Miniprep Kit. Four different cell lines (Cos-7, HEK293, HeLa, and Huh-7) were grown to 80-90% confluence and transfected with 100 ng of each plasmid, in complex with 0.3 µl Lipofectamine 2000, and 10 µl Opti-MEM. Five replicates for each cell type were performed using both DNA preps. GFP expressing cells were counted by flow cytometry 48 hrs post-transfection with a minimum of 2000 events collected per well. Average percentage of cells expressing GFP from all replicates is graphed and used as a measure of transfection efficiency.

ADVANTAGES

- Elute in low volumes
- Prevent buffer retention and salt carryover with optimized column design
- Reduce hands on time with faster protocols and less spin time
- Monitor completion of certain steps using colored buffer system
- No need to add RNase before starting
- Easily label columns using tab and frosted surfaces

SPECIFICATIONS

- **Culture Volume:** 1–5 ml, not to exceed 15 O.D. units
- **Binding Capacity:** up to 20 µg
- **Plasmid Size:** up to 25 kb
- **Typical Recovery:** up to 20 µg, yield depends on plasmid copy number, host strain, culture volume, and growth conditions
- **Elution Volume:** ≥ 30 µl
- **Purity:** $A_{260/280}$ and $A_{260/230}$ ≥ 1.8
- **Protocol Time:** 9½-12½ minutes of spin and incubation time
- **Compatible Downstream Applications:** restriction digestion and other enzymatic manipulations, transformation, transfection of robust cells, DNA sequencing, PCR, labeling, cell-free protein synthesis, etc.

TIPS FOR SUCCESSFUL MINIPREPS

1. **Don't use too many cells (culture should not exceed 15 O.D. units):** Using the optimal amount of cells increases lysis efficiency and ensures that excess cell debris does not clog the column.
2. **Lyse cells completely:** In order to release all plasmid DNA, ALL of the cells need to be lysed. Resuspend cells completely, and incubate for the recommended time.
3. **Don't vortex cells after lysis:** Vortexing can cause shearing of host chromosomal DNA, resulting in gDNA contamination.
4. **Allow the RNase to do its job:** Do not skip or reduce the incubation with RNase (which is included in the neutralization buffer), otherwise you may observe RNA contamination.
5. **Don't skip any washes:** Proper washes ensure the removal of cell debris, endotoxins and salts.
6. **Heat the elution buffer for large plasmids:** Large DNA binds more tightly; heating the elution buffer helps to more efficiently release the DNA from the column matrix.

Please see Page 3 for ordering information and offer details.

A new class of performance

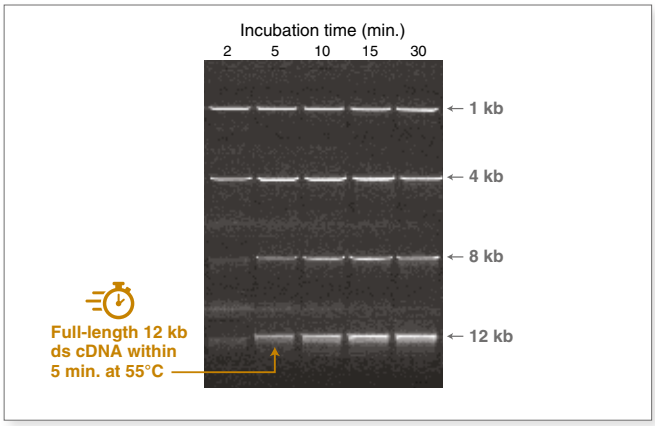
Induro Reverse Transcriptase

Induro Reverse Transcriptase is a group II intron-encoded RT that exhibits high processivity, increased thermostability, and increased tolerance of inhibitors in the synthesis of cDNA from RNA. It is an ideal enzyme for challenging cDNA synthesis from long transcripts, RNAs with strong secondary structures, and RNA samples with inhibitors. With improved 5´ sequencing coverage of long transcripts, Induro Reverse Transcriptase can enable RNA-seq applications such as direct RNA and long read cDNA sequencing workflows.

Benefits

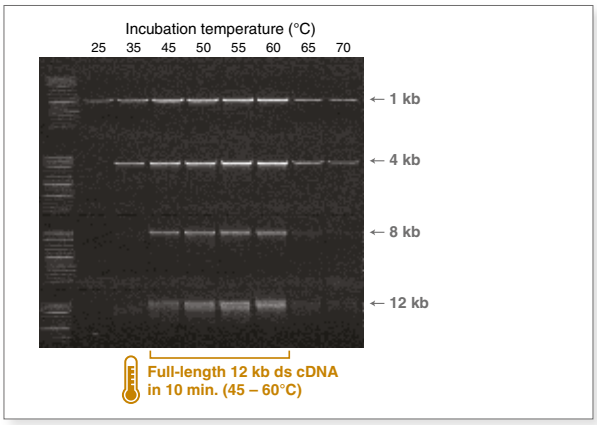
- Rapidly generate high yields of long cDNA with our unique group II intron-encoded RT
- Strong inhibitor tolerance enables robust cDNA synthesis performance
- Support direct RNA sequencing and long read cDNA sequencing workflows
- Generate cDNA at higher temperatures, which is ideal for challenging sample types
- Experience comparable fidelity to retroviral RTs

Induro Reverse Transcriptase exhibits high processivity, permitting rapid cDNA synthesis



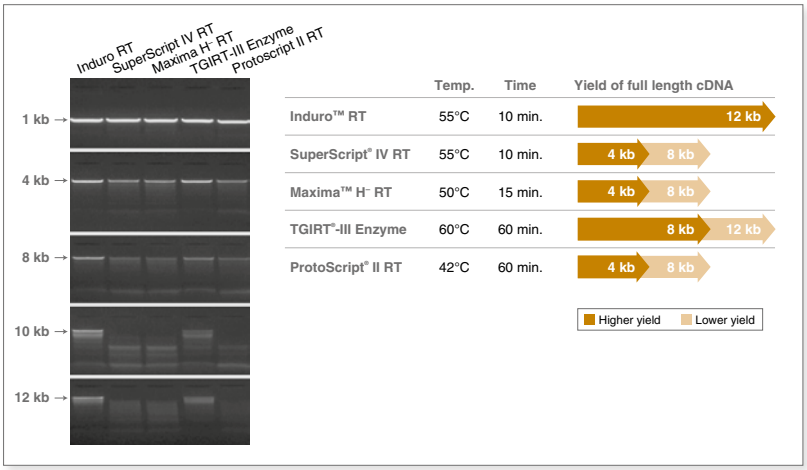
Induro Reverse Transcriptase can synthesize a full-length 12 kb cDNA product within 5 min. at 55°C. In vitro transcribed poly(A) RNA templates (1 kb, 4 kb, 8 kb or 12 kb) were used to investigate full-length cDNA synthesis. After first-strand cDNA synthesis, RNA was hydrolyzed immediately by NaOH. Subsequently, an aliquot of the cDNA products was used to make full-length ds cDNA in the presence of a 5´ specific primer. Equal volume of ds cDNA was analyzed on an agarose gel.

Thermostability of Induro Reverse Transcriptase allows robust synthesis at higher reaction temperatures



Induro Reverse Transcriptase can make a full-length 12 kb cDNA product in 10 min. using incubation temperatures of 45°C to 60°C. In vitro transcribed poly(A) RNA templates (1 kb, 4 kb, 8 kb or 12 kb) were used to investigate full-length cDNA synthesis. After first strand cDNA synthesis, RNA was hydrolyzed immediately by NaOH. Subsequently, an aliquot of the cDNA products was used to make full-length ds cDNA in the presence of a 5´ specific primer. Equal volume of ds cDNA was analyzed on an agarose gel.

Induro Reverse Transcriptase generates highest yields of long cDNA



Induro Reverse Transcriptase generates the highest product yields for cDNA ≥ 8 kb. RNA templates were in vitro transcribed poly(A) RNA (1 kb, 4 kb, 8 kb, 10 kb or 12 kb). After first strand cDNA synthesis, RNA was degraded and the second strand cDNA synthesis was performed in the presence of a 5´ specific primer.

Ordering Information

PRODUCT	NEB #	SIZE
Induro Reverse Transcriptase	M0681S M0681L	4,000 units 10,000 units

For UK pricing please visit www.neb.uk.com

Reverse Transcriptases (RTs) from New England Biolabs

NEB has an extensive portfolio of RTs, which are available as standalone products or have been incorporated into convenient master mixes or kits. For more details and access to the full product listing, visit www.neb.com/rt.

RETROVIRAL RTs

For general cDNA synthesis

LunaScript RT SuperMix
NEB #E3010/M3010

Product notes:
Single-tube supermix, fast 13-minute protocol. Contains random primers, ideal for RNA targets up to 3 kb.

LunaScript® RT Master Mix Kit (Primer-free)
NEB #E3025

Product notes:
First strand cDNA synthesis with user-supplied primers

ProtoScript® II RT
NEB #M0368

Product notes:
RNase H⁻ mutant of M-MuLV RT with increased thermostability and reduced RNase H activity. Ideal for RNA targets up to 12 kb.

ProtoScript II First Strand cDNA Synthesis Kit
NEB #E6560

Product notes:
Kit contains ProtoScript II, Murine RNase Inhibitor, primers and dNTPs

M-MuLV RT NEB #M0253 | **AMV RT** NEB #M0277

Product notes:
Robust RTs for a variety of templates

ProtoScript First Strand cDNA Synthesis Kit
NEB #E6300

Product notes:
Kit contains M-MuLV RT, Murine RNase Inhibitor, primers and dNTPs

For niche cDNA synthesis

LunaScript RT SuperMix
NEB #E3010/M3010

Recommended use:
Two-step RT-qPCR, amplicon sequencing

Luna® WarmStart RT
NEB #M3001*

Recommended use:
One-step RT-qPCR, One-step RT-PCR

Luna One-Step RT-qPCR reagents

- Luna Universal One-Step RT-qPCR Kit
  NEB #E3005
- Luna Universal Probe One-Step RT-qPCR Kit
  ROX: NEB #E3006
  No ROX: NEB #E3007
- Luna Probe One-Step RT-qPCR 4X Mix with UDG
  ROX: NEB #M3019
  No ROX: NEB #M3029
- LyoPrime Luna™ Probe One-Step RT-qPCR Mix with UDG
  NEB #L4001

LunaScript Multiplex One-Step RT-PCR Kit
NEB #E1555

Product notes:
Features Luna WarmStart RT and Q5® Hot Start High-Fidelity DNA Polymerase

Template Switching RT Enzyme Mix
NEB #M0466

Recommended use:
cDNA amplification with a template switching oligo, 5´ RACE, second strand cDNA synthesis

WarmStart® RTx
NEB #M0380

Recommended use:
Optimized for RT-LAMP

LAMP Master Mixes

- WarmStart Colorimetric LAMP 2X Master Mix (DNA & RNA)
  NEB #M1800
- WarmStart Colorimetric LAMP 2X Master Mix with UDG
  NEB #M1804
- WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG)
  NEB #M1708
- WarmStart LAMP Kit (DNA & RNA)
  NEB #E1700
- WarmStart Fluorescent LAMP/RT-LAMP Kit (with UDG)
  NEB #E1708

* Available through OEM & Customized Solutions

GROUP II INTRON RTs

NEW

Induro™ RT
NEB #M0681

Recommended use:

Long cDNA synthesis (up to 20 kb)

Impure RNA samples

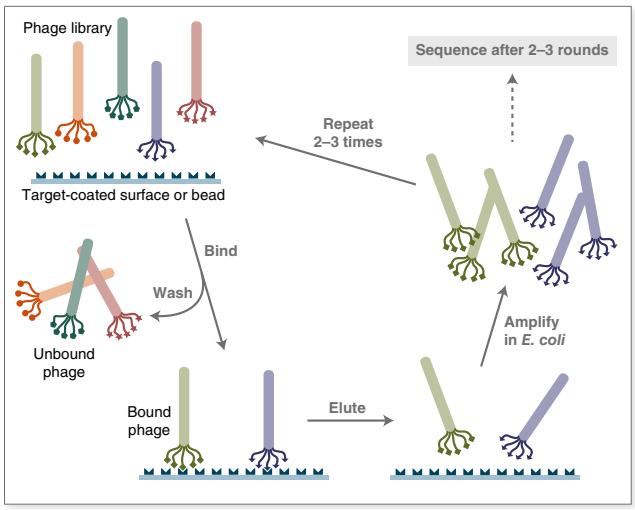
RNA-seq workflows

Product notes:
Group II Intron-encoded RTs are distinct in sequence and domain organization from common retroviral RTs. They exhibit unique features such as high processivity, increased thermostability, and strong inhibitor tolerance.

Ph.D.™ Phage Display Peptide Library Kits v2

The Phage Display Peptide Library Kits v2 contain the Phage Display Peptide Library, a DYKDDDDK Mouse monoclonal antibody and Protein G Magnetic Beads for a panning control experiment, and enough -96gIII sequencing primer for >50 sequencing reactions. The Ph.D. Phage Display Peptide Library is a combinatorial library of random peptides fused to the N-terminus of a minor coat protein (pIII) of M13 phage. The library consists of ~10^9 electroporated (i.e., unique) sequences.

Panning with a pentavalent peptide library displayed on pIII



Benefits

- Ready to use complex phage library (~10^9 clones)
- Panning against DYKDDDDK Mouse monoclonal antibody yields more reliable epitope results than former target protein (streptavidin)
- Does not require helper phage for amplification
- Inherent link between phenotype and genotype allows screening of billions of clones in a single microtiter well or Eppendorf tube

Ordering Information

PRODUCT	NEB #	SIZE
Ph.D.-7 Phage Display Peptide Library Kit v2	E8211S	1 set
Ph.D.-12 Phage Display Peptide Library Kit v2	E8210S	1 set
Ph.D.-C7C Phage Display Peptide Library Kit v2	E8212S	1 set

These products are a direct replacement for NEB #E8100S, Ph.D.-7 Phage Display Peptide Library Kit, NEB #E8110S, Ph.D.-12 Phage Display Peptide Library Kit, and NEB #8120S Ph.D.-C7C Phage Display Peptide Library Kit

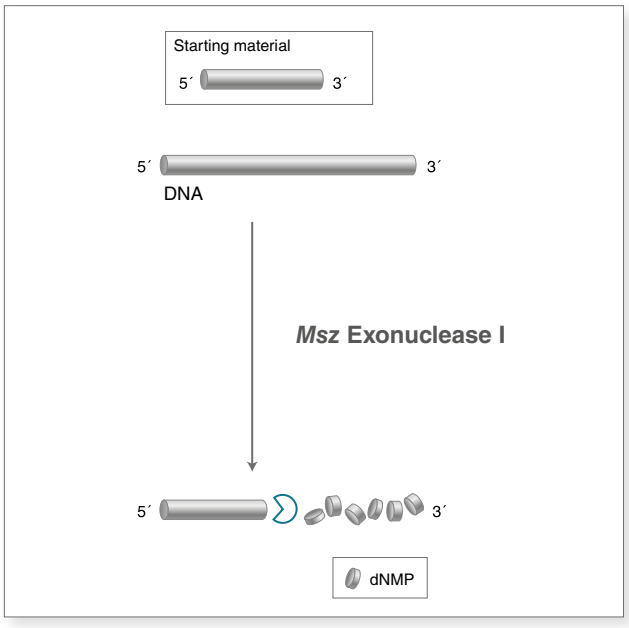
Try a Free Sample of Protein Ladder

Color Prestained Protein Standard, Broad Range (10-250 kDa) (P7719) for high quality Protein Standards with crisp, clear bands.

To learn more and request a free sample, visit
neb.com/forms/proteinladdersample

Msz Exonuclease I

A DNA-specific exonuclease that can be heat inactivated at 80°C in 1 minute



Msz Exonuclease I (*Msz* ExoI) is Ideal for...

- Removal of linear single-stranded DNA oligos in thermophilic workflow applications within 45°C and 60°C.
- This is an Enzyme for Innovation (EFI). EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. These enzymes have interesting properties and unique specificities.

Ordering Information

PRODUCT	NEB #	SIZE
Msz Exonuclease I	M0527S	1000 UNITS

For UK pricing please visit www.neb.uk.com

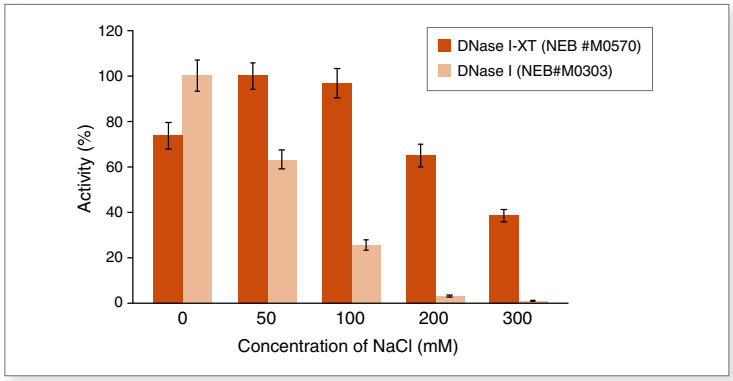
DNase I-XT – Robust removal of DNA

An engineered variant of DNase I, DNase I-XT is a salt-tolerant DNA endonuclease that nonspecifically cleaves DNA to release di-, tri- and oligonucleotide products with 5´-phosphorylated and 3´-hydroxylated ends. DNase I-XT acts on single- and double-stranded DNA, chromatin and the DNA strand of RNA:DNA hybrids. While DNase I (NEB #M0303) is inhibited by salt concentrations > 50 mM, DNase I-XT (NEB #M0570) exhibits optimal activity between 50–100 mM salt and retains 65% and ~40% activity in 200 and 300 mM salt, respectively. This increased salt tolerance makes DNase I-XT the preferred enzyme for DNA template removal from an in vitro transcription (IVT) reaction. Importantly, DNase I-XT is RNase-free, allowing for the complete removal of DNA from RNA preparations while maintaining RNA integrity.

Advantages

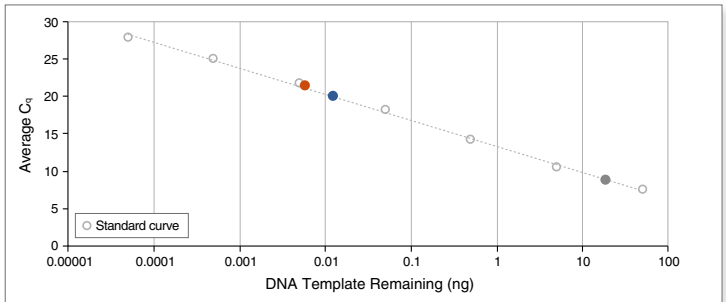
- Use in reactions containing higher amounts of salt, such as IVT and RNA preps
- Add directly to your IVT reaction, with no dilution required
- Efficiently removes DNA from IVT reactions and RNA preps
- RNase-free enzyme tolerates a wide range of salt conditions (up to 300 mM)

DNase I-XT efficiently degrades DNA at salt concentrations > 100 mM



An equimolar comparison of the DNase activity of DNase I (NEB #M0303) and DNase I-XT (NEB #M0570) illustrates the increased salt-tolerance of DNase I-XT. DNase activity was measured by an increase in fluorescence from a quenched 35 nt hairpin dsDNA substrate in 1X DNase I Reaction Buffer with increasing salt concentration (as indicated). While DNase I activity steadily decreases with increasing salt concentrations, DNase I-XT remains active in solutions containing up to 300 mM salt.

Comparison reveals DNase I-XT removes more DNA from IVT reactions and RNA preparations



Sample	Average C_q	DNA Template Remaining (ng)	Residual DNA (%)	Fold DNA Reduction
No DNase	8.83	18.76	–	–
TURBO DNase (2U)	20.14	0.012	0.064	~1,500
DNase I-XT (2U) (NEB #M0570)	21.26	0.006	0.032	~3,000

In vitro transcription reactions (20 µl) were treated with 1) no DNase I; 2) 2 U TURBO™ DNase or 3) 2 U DNase I-XT for 15 minutes at 37°C. Each sample was then purified using the Monarch RNA Cleanup Kit (500 µg, #T2050) and eluted in nuclease-free water (50 µl). The level of residual DNA contamination was quantified by real-time PCR using the Luna Universal Probe qPCR Master Mix (NEB #M3004). Average Cq (quantification cycle) values for each sample were compared to a standard curve (gray) to determine the percent of residual, PCR-amplifiable DNA.

Both TURBO DNase and DNase I-XT require no dilution of the IVT reaction prior to DNase digestion, however, more DNA template is removed from an IVT reaction and undetectable by qPCR when treated with DNase I-XT.

Ordering Information

PRODUCT	NEB #	SIZE
DNase I-XT	M0570S M0570L	1,000 units 5,000 units
COMPANION PRODUCTS		
Monarch RNA Cleanup Kit (500 µg)	T2050S T2050L	10 preps 100 preps
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Multiplex real-time PCR detection of monkeypox virus using Luna® qPCR Reagents

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INTRODUCTION

Monkeypox virus (MPXV) is a double-stranded DNA poxvirus that causes monkeypox, which historically has been a rare disease that results in similar symptoms to smallpox (1). Prior to the 2022 outbreak, monkeypox was mainly found in several countries in Central and West Africa. There are two clades of monkeypox virus, Clade I and Clade II, and infections from the current outbreak stem from Clade II (2). As of October 3, 2022, monkeypox virus has spread to 106 countries with 68,874 cases, resulting in outbreaks in 100 non-endemic countries (3). The WHO Director-General declared the ongoing monkeypox outbreak a Public Health Emergency of International Concern on July 23, 2022. U.S. CDC testing guidance recommends hydrolysis probe-based (e.g., TaqMan®) qPCR for detection of viral targets in DNA purified from patient samples, owing to the high accuracy and sensitivity of qPCR (4,5).

To facilitate molecular diagnostic development efforts, here we demonstrate the detection of synthetic monkeypox viral DNA using Luna qPCR reagents. We show high specificity and sensitivity in the detection of monkeypox DNA using the Luna Universal Probe qPCR Master Mix (NEB #M3004). The limit of detection (LOD) on synthetic viral DNA is five copies per reaction. The exceptional performance in multiplex amplification allows users to detect control human DNA in the same reaction. Similar results were achieved with the Luna Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019), which was evaluated due to the concurrent SARS-CoV-2 pandemic and allows users to rely on a single reagent for detection of both viruses, if desired.

RESULTS

Detecting Monkeypox Using the Luna Universal Probe qPCR Master Mix

Both the non-variola orthopoxvirus generic and monkeypox virus generic single-plex test procedures outlined by the CDC were evaluated using the Luna Universal Probe qPCR Master Mix (NEB #M3004) on three different real-time PCR instruments: Bio-Rad® CFX Opus, Applied Biosystems® (ABI) 7500 and Thermo Fisher Scientific® QuantStudio® 6. Each CDC test procedure requires a minimum of two individual reactions per patient sample: one for non-variola orthopoxvirus or monkeypox virus and a second reaction for human DNA as a control (e.g., RNase P). The non-variola orthopoxvirus single-plex reaction (CDC-OP) includes a pair of primers and a FAM labeled probe that targets a region of the monkeypox virus F8L gene (4). The CDC-OP primer/probe set also detects other orthopoxviruses (e.g., cowpox) except for smallpox, which is caused by variola virus. Although not specific for monkeypox, positive detection using the CDC-OP assay and clinical presentation is sufficient for treatment. The monkeypox virus generic test (MP-Generic) targets the tumor necrosis factor (TNF) receptor gene (J2L) (5,6). However, three recent cases in California have shown a significant deletion in this gene that may lead to false negative results, suggesting caution must be used when using this assay. In our evaluation of these two target designs, the quenchers of both probes were modified according to recommendations provided by the oligo vendor. Additionally, the forward primer for the MP-Generic test was truncated by a single base. Data was collected using our standard concentration recommendations for primer (0.4 µM) and probe (0.2 µM) with slightly modified cycling condition for NEB #M3004 (Table 1). For ABI instruments, the passive reference dye was set to ROX to enable data normalization.

The Luna Universal Probe qPCR Master Mix detected the F8L or J2L gene target from 53,000 copies down to 5.3 copies of synthetic viral DNA template (Synthetic Monkeypox Virus DNA from ATCC #VR-3270SD) on all three instruments with exceptional sensitivity, reproducibility, and qPCR performance using either the non-variola Orthopoxvirus primers/probes or the monkeypox virus generic primers/probes (Figure 1).

Similar performance was observed using the Luna Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019, data not shown). Although this Luna one-step mix is typically intended for RNA detection, the reagent allows higher sample input volumes given its 4X concentration and includes thermolabile UDG for carryover prevention. Given the ongoing SARS-CoV-2 pandemic, this single mix can be used to detect either virus. Furthermore, the CDC's recommendations for primer/probe concentrations and cycling conditions can also be used with either Luna reagent with no observable impact to MPXV detection.

MATERIALS

Reagents

- Luna® Universal Probe qPCR Master Mix (NEB #M3004)
  or
  Luna Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019)

Primers and probes

- See Table 1

Template

- ATCC® Quantitative Synthetic Monkeypox virus DNA (#VR-3270SD)

Sensitive Detection of Monkeypox Using the Luna Universal Probe qPCR Master Mix in 2-plex assay

Multiplex assays offer a more efficient testing option in diagnostic settings, allowing a single sample to be interrogated for various targets simultaneously. Each monkeypox CDC test design described above utilizes detection of RNase P in a second independent reaction as a control to confirm the presence of human DNA. We investigated the use of a 2-plex assay that allows for detection of human RNase P and the monkeypox virus F8L gene (CDC-OP) in a single reaction. The FAM fluorophore of the human RNase P probe was changed to Cy5™ to accommodate the multiplex design. This allows detection of the internal control in the Cy5 channel while non-variola Orthopoxvirus detection remains in the FAM channel.

The Luna Universal Probe qPCR Master Mix detected the monkeypox F8L target from 53,000 copies down to 5.3 copies of synthetic viral DNA template (ATCC #VR-3270SD) in the presence of 5 ng Jurkat DNA on all 3 instruments (Figure 2), consistent with the performance in the single-plex

TABLE 1: **Primers and Probes**

Cycling conditions (NEB recommendation): Single-plex or 2-plex: 95°C for 1 min., 45 cycles of 95°C for 10 sec., 60°C for 30 sec.
PCR instruments: Bio-Rad CFX Opus (Default mode), Applied Biosystems (ABI) 7500, Thermo Fisher Scientific QuantStudio 6

ASSAY	PRIMER/PROBE		REF.	NOTE	CONC. (µM)
	NAME	SEQUENCE			
CDC-Non-variola Orthopoxvirus	CDC-OP-F	5´-TCAACTGAAAAGGCCATCTATGA-3´	CDC	Probe quencher modified	Primer: 0.4 Probe: 0.2
	CDC-OP-R	5´-GAGTATAGAGCACTATTTCTAAATCCCA-3´			
	CDC-OP-FAM	5´-FAM-CCATGCAAT/ZEN/ ATACGTACAAGATAGTAGCCAAC-3´IABkFQ			
RP-DNA	RP-DNA-F	5´-AGATTTGGACCTGCGAGCG-3´	CDC	Probe fluorescent dye and quencher modified	Primer: 0.4 Probe: 0.2
	RP-DNA-R	5´-GAGCGGCTGTCTCCACAAGT-3´			
	RP-DNA-Cy5	5´-Cy5-TTCTGACCT/ZEN/GAAGGCTCTGCGCG-3´IABkFQ			
MP-Generic	MP-G-F	5´-GGAAAATGTAAAGACAACGAATACA-3´	CDC	F primer and probe quencher modified	Primer: 0.4 Probe: 0.2
	MP-G-R	5´-GCTATCACATAATCTGGAAGCGTA-3´			
	MP-G-FAM	5´-FAM-AAGCCGTAA/ZEN/TCTATGTTGTCTATCGTGTCC-3´IABkFQ			

FIGURE 1: **The Luna Universal Probe qPCR Master Mix offers exceptional sensitivity, reproducibility and qPCR performance**

Detection of the monkeypox F8L gene using the CDC-OP primers/probe (A) and the J2L gene using the CDC MP-G primers/probe (B) was performed using the Luna Universal Probe qPCR Master Mix over a 5-log range of input template (53,000 cp – 5.3 cp synthetic monkeypox virus DNA, ATCC #VR-3270SD) with 2 replicates at each dilution. Instruments: CFX Opus (Bio-Rad®), ABI 7500 and QuantStudio 6 (Thermo Fisher Scientific®).

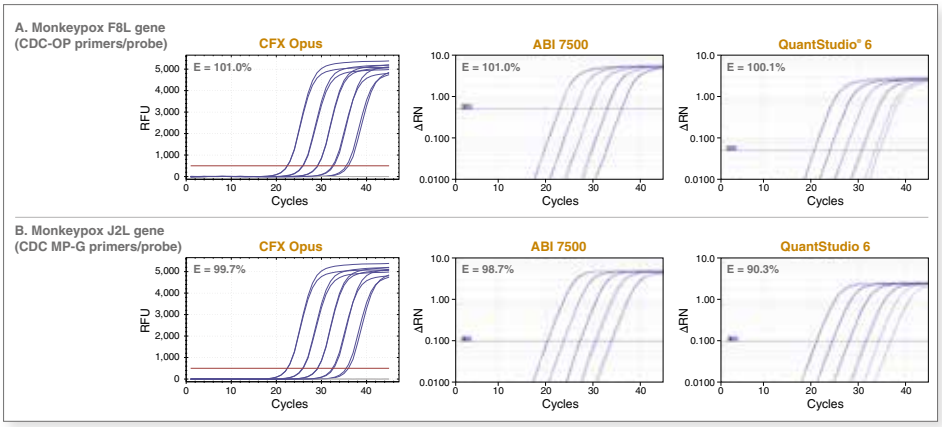


FIGURE 2: **The Luna Universal Probe qPCR Master Mix offers robust performance in 2-plex detection of monkeypox and human RNase P control DNA**

2-plex qPCR assays targeting the monkeypox F8L gene (CDC-OP, blue) and human RNase P DNA (RP-DNA, light purple) were performed using the Luna Universal Probe qPCR Master Mix over a 5-log range of input template (53,000 cp – 5.3 cp synthetic monkeypox virus DNA, ATCC #VR-3270SD, diluted in 5 ng/µl Jurkat genomic DNA) with 2 replicates at each dilution. To verify the limit of detection (LOD) of the Luna qPCR reagent, an additional 34 replicates of 5 cp input was tested in the 2-plex assay. Across all three instruments, 34 out of 34 replicates were detected. Instruments: CFX Opus (Bio-Rad), ABI 7500 and QuantStudio 6 (Thermo Fisher Scientific).

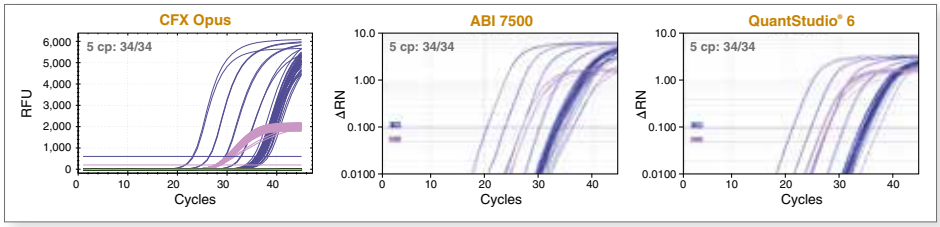
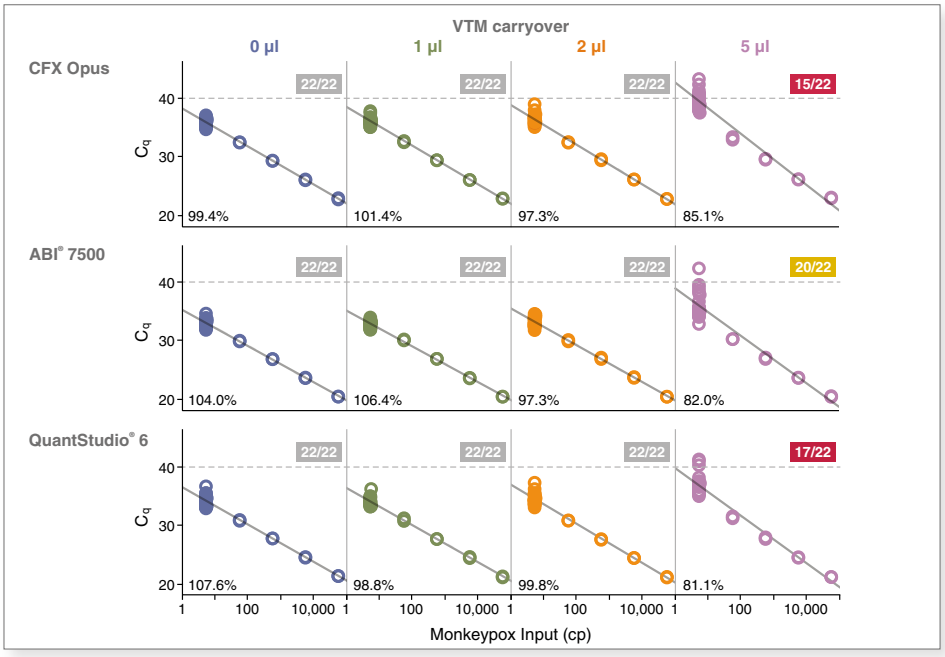


FIGURE 3: **Luna Universal Probe qPCR Master Mix tolerates up to 10% Viral Transport Medium**

2-plex qPCR assays targeting the monkeypox F8L gene (CDC-OP) and human RNase P (RP-DNA) were performed using the Luna Universal Probe qPCR Master Mix over a 5-log range of input template amount (53,000 cp – 5.3 cp synthetic monkeypox virus DNA, ATCC #VR-3270SD in 10 ng of Jurkat RNA) with 2 replicates at each dilution. To test any impact to LOD, an additional 22 replicates of 5 cp input was tested in the 2-plex assay. The assays were performed in the absence or presence of a gradient of Viral Transport Medium (VTM) up to 5 µl in a 20 µl reaction. Instruments: CFX Opus (Bio-Rad), ABI 7500 and QuantStudio 6 (Thermo Fisher Scientific).



assay. To determine the LOD (95% confidence) of the monkeypox 2-plex assay, we evaluated the Luna reagent on all three real-time PCR instruments. The LOD for each instrument was established by testing 34 replicates of 5-copy synthetic viral DNA input and 2 non-template controls. The Luna Universal Probe qPCR Master Mix detected all 34 replicates on all three qPCR instruments, while the non-template control reactions lacked amplification. RNase P was also 100% detected simultaneously on Cy5 channel. Similar results were also observed using the Luna Probe One-Step RT-qPCR 4X Mix with UDG.

Reagent Tolerance to Viral Transport Medium (VTM)

Currently, both the WHO and the CDC recommend specimen collection from skin lesion swabs and DNA extraction for diagnostic testing. However, extraction-free workflows may be possible. Given that swabs can be stored dry or in Viral Transport Medium (VTM), VTM tolerance is critical for development of direct detection workflows of monkeypox virus DNA in patient samples. We therefore tested the effects of VTM on the Luna Universal Probe qPCR Master Mix.

The Luna reagent was highly tolerant of VTM, with no detectible effect on quantitation and LOD detection with 2 µl VTM presence per 20 µl reaction (10% v/v) (Figure 3). Detection of low input (5 copies/reaction) was impacted by 5 µl VTM per 20 µl reaction (25% v/v), but negligible effects were observed for high inputs.

CONCLUSION

Molecular diagnostics continue to play a critical role in the detection and diagnosis of infectious diseases. The recent rise in monkeypox cases around the globe has sparked concern and many labs are turning to nucleic acid amplification tests to help prevent the spread of the disease. The details outlined herein showcase how the Luna reagents can be used in previously developed single-plex real-time PCR assays by the CDC for detection of monkeypox virus while achieving an LOD of 5 copies of synthetic DNA per reaction. Modification of the fluorophores and use of the Luna reagents allows these real-time PCR assays to be converted into multiplex tests. We hope the high specificity and sensitivity data and recommendations presented will help labs develop monkeypox assays that meet their specific needs.

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