NEWS on diagnostics

2024 Volume 3

Welcome to this final edition of News on Diagnostics for 2024.

This volume highlights some of the key products and reagents MilliporeSigma has available to create a robust and reliable diagnostic assay.

In this issue....

Molecular Diagnostics Workflows

PCR (Polymerase Chain Reaction) revolutionized molecular biology and genetics. Its ability to amplify small amounts of DNA made it an indispensable tool in various fields.

In clinical diagnostics, PCR is used to detect pathogens, identify genetic mutations, and monitor disease progression. In research, it's crucial for cloning genes, sequencing DNA, and conducting quantitative studies like qPCR, which allows for the measurement of DNA or RNA levels in real-time.

The Human Genome Project is a prime example of PCR's impact, enabling the sequencing of the entire human genome. This has paved the way for advancements in personalised medicine, genomics, and biotechnology.

Each individual step of the assay workflow is just as important as the next, as it creates a clean and accurate 'sample' for the next stage. Even small errors within a step can be augmented in the next stage, and, overall, create an assay with poor repeatability and reproducibility, rendering it inadequate for diagnostic purposes.

Using high quality reagents from MilliporeSigma at critical stages of the assay supports performance and efficacy.

Benchtop workflow at R+D level for DNA and RNA purification and amplification, but where an initial amplicon was generated using dUTP: GenElute®-E – Heat-labile Cod Uracil-DNA Glycosylase - Glycerol-free - JumpStart™ Taq DNA Polymerase

Purification of viral particles from saliva, saline, Amies medium, and viral transport at an R+D level (but could also be scalable) for RNA purification and amplification, but where an initial amplicon was generated using dUTP: Viral RNA Extraction Buffer - Heat-labile Cod Uracil-DNA Glycosylase.

Instrument-based or manual recovery of DNA and RNA from biological sample (scalable), but where an initial amplicon was generated using dUTP: (ProteinaseK/lysis buffer) – MagPrep™ Silica –Heat-labile Cod Uracil-DNA Glycosylase - Glycerol-free JumpStart™ Taq DNA Polymerase.

Product Spotlights:

GenElute™ E

The GenElute™-E Single Spin Kits represent a significant advancement in nucleic acid purification. They simplify the process by employing a negative chromatography method based on size exclusion, which effectively separates large DNA and RNA molecules from smaller impurities such as proteins, lipids, and ions. This method eliminates the need for high salt binding and ethanol wash steps, which are common in traditional silica-based methods. As a result, the DNA and RNA preparations are purer and more suitable for sensitive downstream applications.

These kits not only provide high-quality nucleic acid samples but also align with the principles of green chemistry, contributing to a more sustainable laboratory environment via

- Waste prevention
- Better usability through a simplified workflow
- Sustainable packaging
- Safer disposal

Try GenElute™-E Single Spin purification, **request a sample!**

Discover how GenElute™-E Single Spin technology enables nucleic acid purification in a single step! **Download our infographic now.**

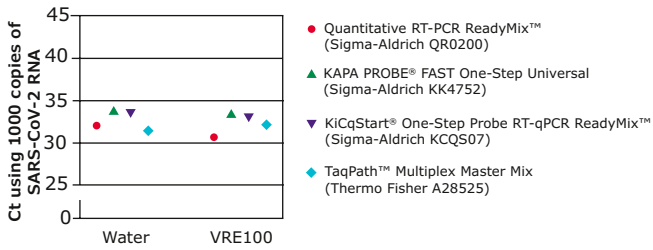
Viral RNA Extraction Buffer

The Viral RNA Extraction Buffer simplifies the process of testing and analysis respiratory viruses, by eliminating the need for RNA purification steps, which can be time-consuming and resource-intensive. The buffer's ability to lyse viral particles and stabilise RNA at room temperature makes it particularly useful for rapid testing environments.

The Viral RNA Extraction Buffer is broadly compatible with most nucleic acid detection methods and does not reduce signal when used at working concentration in qRT-PCR. qRT-PCR compatibility has been demonstrated in a 4-fluor multiplex setting consisting of Cy5, TxRed, FAM, and HEX.

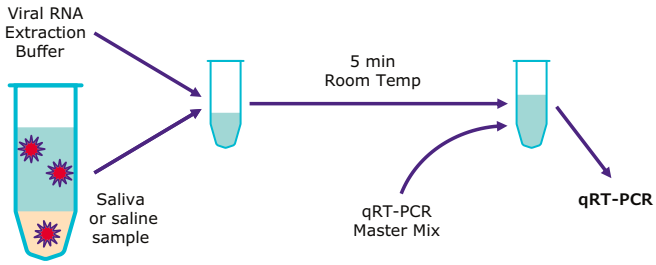
RT-qPCR compatibility has been verified with several one-step RT-qPCR kits:

- Quantitative RT-PCR ReadyMix™ (Sigma-Aldrich, catalog QR0200)
- KAPA PROBE FAST One-Step Universal (Sigma-Aldrich, catalog KK4752)
- KiCqStart® One-Step Probe RT-qPCR ReadyMix™ (Sigma-Aldrich, catalog KCQS07)
- TaqPath™ 1-Step Multiplex Master Mix (Thermo Fisher, catalog A28525)



The Viral RNA Extraction Buffer is also compatible with colorimetric LAMP assays.

MagPrep® Silica



MagPrep Silica refers to a type of silica-coated magnetic particles used in the purification of nucleic acids.

Some key features and benefits of MagPrep Silica include:

High Efficiency: They will bind RNA as well as DNA with very high efficiency.

Versatility: Suitable for a variety of applications, requiring nucleic acid purification from a range of sample types.

Rapid Processing: Allows for quick magnetic separation (<15s), useful for high-throughput screening applications.

Particle Size: The particle size range of 100-200nm ensures a large surface area for nucleic acid binding.

Stability: Homogenised suspensions are stable for up to five minutes without further agitation; beneficial during the processing of multiple samples.

No Ethanol Required: simplifying the process and reducing the risk of contamination.

Easy Elution: Bound nucleic acids can be eluted with any aqueous buffer (pH >8.0, making the process straightforward.

MagPrep® Silica LS is particularly useful for laboratories looking to automate the nucleic acid purification process while maintaining high throughput and efficiency.

Heat-labile Cod Uracil-DNA Glycosylase

Heat-labile Cod Uracil-DNA Glycosylase (UNG or UDG) is an enzyme used in PCR and other nucleic acid amplification techniques to prevent carry-over contamination from previous reactions.

Uracil-DNA Glycosylase excises uracil from dU-containing DNA by cleaving the N-glycosidic bond between the uracil base and the sugar backbone.

This version of UDG from *Gadus morhua* (cod) is heat-labile, meaning it can be completely and irreversibly inactivated at a moderate temperature (e.g., 10 minutes at 50 °C), which is beneficial for some PCR protocols.

It's used to increase cloning efficiency of PCR products and the efficiency of site-directed mutagenesis. It's also useful for studying DNA repair and mutation detection.

The heat-labile property ensures that the enzyme does not interfere with the PCR product post-amplification, making it suitable for downstream applications like cloning, sequencing, or genotyping.

Despite being heat-labile, the enzyme is stable and active until its intentional inactivation. It's typically stored in a buffer containing glycerol, but for the heat-labile version, storage conditions may vary.

This enzyme is particularly useful in applications where carry-over contamination could be a problem and where subsequent analysis of the PCR product is necessary.

Glycerol-free JumpStart™ Taq DNA Polymerase

The blend of our high-performance Taq DNA Polymerase and JumpStart™ Taq antibody that is suitable for lyophilization due to the lack of the commonly used stabiliser, glycerol. It allows the user to lyophilise PCR master mixes, which can be transported and stored at ambient temperature for a prolonged time.

Glycerol-free formulations are also often used in applications such as dry-down PCR, where the reagents are dried into the wells and rehydrated before use.

Despite the lack of glycerol, this enzyme maintains high performance in PCR reactions, providing the same benefits of hot-start PCR to improve specificity and yield.

Overall, hot-start PCR enhances the specificity and yield of the PCR process, making it a valuable method for applications where precision is crucial, such as cloning, sequencing, and diagnostic testing etc by reducing background noise from non-specific amplification and increasing the clarity of the desired bands on a gel.

This enzyme formulation is combined with an antibody that inactivates it at room temperature, and it becomes active only when the temperature is raised during PCR cycling.

Antibody-mediated hot start

- Designed to minimize non-specific amplification.
- Increased specificity/yield, reduced primer-dimers
- Room-temperature reaction set-up with reduced set-up time

Glycerol-free enzyme that is lyophilization-compatible.

- Extended shelf life: Removing water increases the shelf life & stability.
- Easy handling & transportation reduces the risk of damage and loss of activity during shipping.
- Removal of water means reduced chances of contamination.
- Lyophilized enzyme can be easily reconstituted prior to your assay.

Quality

The Quality Segments of the M-Clarity™ Program provide transparency so that you can choose, with confidence, suitable products that meet your needs with respect to:

- Compliance with the appropriate quality and regulatory standards
- Portfolio transparency
- Change notification service
- Documentation and support

The table on the right shows the classification of the products mentioned in this newsletter. For more information about the M-Clarity™ Quality Program, and the attributes associated with each MQ segment, please visit **SigamaAldrich.com/mclarity** Usage of all or any of these products is subject to due diligence and risk assessment by the user.

Product Number	Product Description	MQ Segment	Change Notification available?	Add to Quality Agreement?
D9310	Glycerol-free JumpStart™ Taq DNA Polymerase	MQ200	Limited	No
SRE0111	Heat-labile Cod Uracil-DNA Glycosylase	MQ200	Limited	No
SRE0112	Heat-labile Cod Uracil-DNA Glycosylase	MQ200	Limited	No
VRE100	Viral RNA Extraction Buffer	MQ200	Limited	No
Various	GenElute™ -E kits	n/a	n/a	n/a
1.01193	MagPrep® Silica	MQ300	Yes	Yes

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