For general laboratory use.

Print Changes to Previous Version | Print Safety Precautions | Print

High Pure Viral Nucleic Acid Buffer Set

Version: 12
Content version: September 2017

For the isolation of viral nucleic acids for PCR or RT-PCR

Cat. No. 12 011 875 001 1 set
up to 100 isolations

Store the set at +15 to +25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	green	Lysis/Binding Buffer	Contains 6 M guanidine-HCl, 10 mM urea, 10 mM Tris-HCl, 20% Triton X-100 (v/v), pH 4.4 (+25°C)	▪ 25 ml
2	white	Poly(A)	Lyophilizate for binding of RNA	▪ 2 mg poly(A) carrier RNA
3	pink	Proteinase K	Lyophilizate for the digestion of proteins	▪ 100 mg
4a	black	Inhibitor Removal Buffer	Contains 5 M guanidine-HCl, 20 mM Tris-HCl, pH 6.6 (+25°C) (final concentration after addition of ethanol)	▪ 33 ml, add 20 ml absolute ethanol
4	blue	Wash Buffer	Contains 20 mM NaCl, 2 mM Tris-HCl, pH 7.5 (+25°C) (final concentrations after addition of ethanol)	▪ 20 ml add 80 ml absolute ethanol
5	colorless	Elution Buffer	Water PCR grade	▪ 30 ml

⚠ ***All solutions are clear, except Vial 1 Lysis / Binding Buffer which is clear to slightly turbid, and should not be used when precipitates have formed. Warm the solutions at +15 to +25°C or in a 37°C water bath until the precipitates have dissolved.***

ⓘ *The buffers can show a slight yellow color. This will have no impact on the function of the buffer.*

1.2. Storage and Stability

Storage Conditions (Product)

⚠ ***The High Pure Viral Nucleic Acid Buffer Set components must be stored at +15 to +25°C. If properly stored, all kit components are stable until the expiration date printed on the label.***

⚠ ***Improper storage at +2 to +8°C (refrigerator) or −15 to −25°C (freezer) may lead to formation of salt precipitates in the buffers which will adversely impact the performance of the kit.***

Storage Conditions (Working Solution)

Solution	Storage
Proteinase K	−15 to −25°C
poly(A) carrier RNA	−15 to −25°C

1.3. Additional Equipment and Reagents Required

- High Pure Viral Nucleic Acid Kit*
- Absolute ethanol
- Isopropanol
- Microplate centrifuge capable of a 1,800 × *g* centrifugal force
- Standard laboratory equipment

1.4. Application

This buffer set supplies additional buffers thereby increasing the flexibility of the High Pure Viral Nucleic Acid Kit*.

1.5. Preparation Time

Total time	Approx. 20 minutes
Hands-on time	<10 minutes

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Samples as specified in the Instructions of Use of the High Pure Viral Nucleic Acid Kit.

⚠ ***Samples containing precipitates must be centrifuged before purification.***

General Considerations

Handling Instructions

⚠ ***Guanidine-hydrochloride in Lysis / Binding Buffer and Inhibitor Removal Buffer is an irritant. Always wear gloves and follow standard safety precautions to minimize contact when handling.***
⚠ ***Do not allow these buffers touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water; otherwise, the reagent may cause burns. If you spill the reagent, dilute the spill with water before wiping it up.***
⚠ ***Never store or use the Binding Buffer near human or animal food.***
⚠ ***Always wear gloves and follow standard safety precautions when handling these buffers.***
⚠ ***Use sterile disposable polypropylene tubes and tips to avoid RNase contamination. Always wear gloves during the assay.***
⚠ ***Do not allow the Lysis/Binding or Inhibitor Removal buffers to mix with sodium hypochlorite found in commercial bleach solutions. This mixture can produce a highly toxic gas.***

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Do not contaminate the reagents with bacteria, virus, or nucleases. Use disposable pipets and nuclease free pipet tips only, to remove aliquots from reagent bottles. Use the general precautions described in the literature.
- Wash hands thoroughly after handling samples and reagents.
- Finish each phase of the PCR/RT-PCR workflow before proceeding to the next phase. For example, you should finish PCR/RT-PCR sample preparation before starting PCR/RT-PCR set-up. Sample preparation, PCR/RT-PCR setup and the PCR/RT-PCR run itself should also be performed in separate locations.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

In addition to the ready-to-use solutions supplied with this kit, prepare the following working solutions:

Content	Reconstitution / Preparation	Storage / Stability
poly(A) carrier RNA (Vial 2; white cap)	① Add 0.5 ml Elution Buffer to the Poly(A) carrier RNA. ② Add stopper and invert the vial until all the carrier RNA (including any that might stick to the rubber stopper) is completely dissolved. ③ Aliquot the reconstituted carrier RNA according to the following table depending on your preferred sample volume and numbers into separate nuclease-free microcentrifuge tubes.	▪ Store at −15 to −25°C ▪ Stable for 12 months
Proteinase K (Vial 3, pink cap)	① Dissolve Proteinase K in 5 ml Elution Buffer. ② Aliquot the reconstituted Proteinase K according to the following table depending on your preferred sample volume and numbers into separate nuclease-free microcentrifuge tubes.	▪ Store at −15 to −25°C ▪ Stable for 12 months
Inhibitor Removal Buffer (Vial 4a; black cap)	Add 20 ml absolute ethanol to Inhibitor Removal Buffer and mix well. ⚠ ***Label and date bottle accordingly after adding ethanol.***	▪ Store at +15 to +25°C. ▪ Stable until expiration date printed on kit label.
Wash Buffer (vial 4; blue cap)	Add 80 ml absolute ethanol to Wash Buffer and mix well. ⚠ ***Label and date bottle accordingly after adding ethanol.***	▪ Store at +15 to +25°C. ▪ Stable until expiration date printed on kit label.
Working solution	The composition of the working solution is independent of the sample volume. Poly(A) carrier RNA dissolved in Binding Buffer is not stable. ⚠ ***The working solution (Binding Buffer with Poly(A) and Proteinase K) must be prepared freshly before each use.***	▪ Use immediately

Amount of working solution required for varying sample volumes:

If you intend to purify....		THEN prepare the following amount of working solution freshly before each experiment...				
Sample volume [µl]	Number of samples	Binding Buffer (Vial 1, green cap) [µl]	Recon-stituted Poly(A) [µl]	Recon-stituted Proteinase-K [µl]	Volume of working solution per sample [µl]*	Maximum number of samples per High Pure Viral Nucleid Acid Buffer Set
200	1	250	5	50	250	100
	12	3000	60	600		
300	1	375	7.5	75	375	66
	12	4500	90	900		
400	1	500	10	100	500	50
	12	6000	120	1200		
600	1	750	15	150	750	33
	12	9000	180	1800		

* Maximum capacity of the High Pure Filter tube is 700 µl. When processing samples volumes larger than 300 µl, load the Filter Tubes multiple times.

3. Results

Experimental Results

Each preparation was used as a template in PCR (DNA) or RT-PCR (RNA). All these templates produced highly specific PCR products with good yield and sensitivity.

4. Troubleshooting

Observation	Possible cause	Recommendation
Low nucleic acid yield or purity	Buffer set stored under non-optimal conditions.	Store the set at +15 to +25°C at all times upon arrival.
	Buffers or other reagents were exposed to conditions that reduced their effectiveness.	Store all buffers at +15 to +25°C.
		Close all reagent bottles tightly after each use to preserve pH, stability, and freedom from contamination.
		After any lyophilized reagent is constituted, aliquot it and store the aliquot at −15 to −25°C.
	Ethanol not added to Wash Buffer and Inhibitory Removal Buffer	① Add absolute ethanol to the buffers before using. ② After adding ethanol, mix the buffers well and store at +15 to +25°C. ③ Always mark Wash Buffer vial and Inhibitory Removal Buffer vial to indicate whether ethanol has been added or not.
	Reagents and samples not completely mixed.	Always mix the sample tube well after addition of each reagent.
Poor elution of nucleic acids with water	Water has the wrong pH	If you use your own water or buffer to elute nucleic acids from Filter Tube, be sure it has the same pH as the Elution Buffer supplied in the kit.
Absorbance ($A_{260\ nm}$) reading of product too high	Glass fibers, which might coelute with nucleic acid, scatter light	① Spin Elution Rack for 1 minute at maximum speed. ② Transfer supernatant into a new tube without disturbing the glass fibers at the bottom of the original tube.
Low RNA yield	High levels of RNase activity	Be careful to create an RNase-free working environment.
		Process starting material immediately or store it at −60 to −80°C until it can be processed.
		Use eluted RNA directly in downstream procedures or store it immediately at −60 to −80°C.
	Incomplete Proteinase K digestion	Be sure to dissolve the lyophilized Proteinase K completely as follows: ① Pipet 5 ml of Elution Buffer into the glass vial containing lyophilized Proteinase K. ② Stopper and invert the vial until all the lyophilizate (including any stuck to the rubber stopper) is dissolved. ③ Aliquot the reconstituted enzyme, mark each aliquot with the date of reconstitution, and store at −15 to −25° C. ⚠ ***Reconstituted Proteinase K is stable for 12 months when stored properly.***

5. Additional Information on this Product

5.1. Test Principle

The High Pure Viral Nucleic Acid Buffer Set is intended to be used in combination with the High Pure Viral Nucleic Acid Kit. The kit is designed for the purification of viral nucleic acids from serum or plasma. Nucleic acids can be applied in PCR or RT-PCR directly after elution in nuclease-free water. The purification procedure uses filter tubes instead of extraction with organic solvents or nucleic acid precipitation steps.
The samples are lysed by incubation with a special buffer containing Proteinase K and guanidine hydrochloride that releases nucleic acids (Cory, S. et al., 1983). A highly efficient reaction is obtained at elevated temperatures. Subsequently, the liquid is centrifuged through a glass fiber filter that is contained in the kit (Yang, R. et al., 1979). During this process, the nucleic acids are bound specifically to the surface of the glass fiber (Jakobi, R. et al., 1988; Kristensen, T. et al., 1987). Unbound substances are removed by centrifugation. The absorbed nucelic acids are washed and eluted with an aqueous solution.

① The isolation of the analyte from serum or plasma is required. Virus lysis is accomplished by incubating the sample in a special Lysis / Binding Buffer.

② Isolation of the nucleic acids by binding to the glass fibers.

③ Washing of bound nucleic acids, purification from salts, proteins and other cellular impurities.

④ Elution of the purified NA with Elution Buffer.

5.2. References

- Cory S, Gerondakis S, Adams JM - Interchromosomal recombination of the cellular oncogene c-myc with the immunoglobulin heavy chain locus in murine plasmacytomas is a reciprocal exchange. (1983) *EMBO J* **5**, 697-703
- Jakobi R, Wiemann S, Pyerin W - Filter-supported preparation of lambda phage DNA (1988) *Analytical Biochemistry* **1**, 196-201
- Kristensen T, Voss H, Ansorge W - A simple and rapid preparation of M13 sequencing templates for manual and automated dideoxy sequencing (1987) *Nucleic Acids Research* **14**, 5507-5516
- Yang RC, Lis J, Wu R - [10] Elution of DNA from agarose gels after electrophoresis (1979) , 176-182

5.3. Quality Control

Series of MS 2 RNA dilution are prepared, applied to the filter tubes, washed and eluted following the kit protocol. 3.5 µl of the eluate is analyzed by RT-PCR.
The products are detected on agarose gel. At least 2 × 10^5 RNA molecules/200 µl sample are guaranteed.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout Changes.
Editorial Changes.

6.3. Ordering Information

Roche offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our homepage lifescience.roche.com.

Product	Pack Size	Cat. No.
Reagents , kits		
High Pure Viral Nucleic Acid Kit	1 kit, up to 100 isolations	11 858 874 001

6.4. Trademarks

HIGH PURE is a trademark of Roche.
All third party product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

For patent license limitations for individual products please refer to: http://technical-support.roche.com.

6.6. Regulatory Disclaimer

For general laboratory use.

6.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.8. Contact and Support

If you have questions or experience problems with this or any Roche product for Life Science, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help.
Please also contact us if you have suggestions for enhancing Roche product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to the research community worldwide.

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support** Site.

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0917.12036053001 (12)

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