DTPM®

Cyclone DNA/RNA Purification Kit

— User Guide —

REF EXT52420 Version 1.0

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For Research Use Only
Not For Use In Diagnostic Procedures

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PRODUCT DESCRIPTION

Kit Description

The DTPM Cyclone DNA/RNA Purification Kit is designed as a fast, efficient, and cost-effective solution for users who require high quality, high yield nucleic acid purification.

Each kit contains silica membrane spin columns, lysis solution, wash buffers, and high-purity elution buffer yielding isolated DNA/RNA suitable for PCR, qPCR, and most NAAT applications.

Contents and Storage

Store each kit component according to the storage recommendations in the table below. Wash buffer 1 and wash buffer 2 are stable until expiration dates following the addition of ethanol to each concentrate.

Material Name	Volume/Quantity	Recommended Storage Condition
Column Preparation Liquid	10 x 1.4 mL	15 – 25º C
Lysis Solution [a]	5 x 12 mL	
Wash Buffer 1[a] (concentrate)	5 x 25 mL	
Wash Buffer 2 (concentrate)	5 x 11 mL	
Eluent	20 x 1.25 mL	
Proteinase K	10 x 1.3 mL	2 – 8º C
Carrier RNA	5 vials	– 20º C
Wash Tubes	20 x 50 tubes	15 – 25º C
Spin Columns	5 x 50 tubes (250 preps)	

[a] Contains guanidine hydrochloride (guanidinium chloride)

METHODS

Important Guidelines

Inspect the content of the kit to ensure the integrity of included components upon receipt. Do not use damaged products.

Refer to Safety Data Sheets (SDS) for precautions related to material handling. Always wear personal protective equipment (PPE) when handling reagents. The included Lysis Solution and Wash Buffer 1 contain potential irritants and must be handled with appropriate precautions.

Treat all sample and waste as potentially infectious. Avoid contact with skin and eyes when handling sample materials and reagents.

Always follow appropriate waste handling and disposal guidelines as designated by local authorities. Disinfect work areas which may be exposed to sample materials and/or reagents.

Always use RNase-free equipment and reagents.

Refer to your laboratory safety guidelines for additional information or restrictions related to use of these materials.

Sample Handling

- Store samples according to established guidelines for each collection device and sample type.
- Avoid multiple freeze/thaw cycles to ensure nucleic acid integrity.
- Equilibrate samples to room temperature (15º C – 25º C) before use.
- Treat all samples as potentially infectious materials.
- Whenever possible, use fresh sample material.

Materials Required not provided

Unless otherwise indicated, all materials are available through DTPM. Please visit **store.DTPM.com** or contact your **customer representative** for further information.

Material Name	Product Number
Disposable Sampling Tube – MTM	PV990001
Molecular Grade Water	SH3053802
Molecular Grade Ethanol 200 proof (100%)	04-355-450
Phosphate Buffered Saline (PBS)	70-011-044 FH
RNAse Away	21-402-178
2.0 mL Screw Cap Microcentrifuge Tube (optional)	1420-9710 US
E1-ClipTip™ Bluetooth™, Yellow, 2-125 µL, Multichannel Equalizer Pipette	14-3879-74BT FH
Research® plus Adjustable Volume Pipettes 0.5 – 10 µL, 10 – 100 µL, 100 – 1000 µL	14-285-904 FH
Pipette tips, sterile, filtered, 10 µL	10uL-XL-SFR
Pipette tips, sterile, filtered, 20 µL	DTPM-20uL 01
Pipette tips, sterile, filtered, 100 µL	100uL-SFR
Pipette tips, sterile, filtered, 200 µL	200uL-SFR
1000-1250 µL Pipette Tips, Racked with Filter, PP, DNase and RNase-Fre, Sterile, 96 Tips/Rack - 50 Racks	1250uL-SFR
384-well, 40 µL, full skirt, clear, sterile	384-40-FSC
Optical Adhesive PCR Film	PA990001
FreeWipes Lint Free Wipes	YA990011
Vortex-Genie® 2, 120V	50-728-002 FH
Nitrile Gloves	PRO100113-S, PRO100113-M, PRO100113-L, PRO100113-XL

Before Use of the Cyclone Kit

General Guidelines

- Read all instructions prior to use.
- Inspect the kit and all material packaging to ensure the contents are present and in good condition.
- Be sure to review all of the required equipment and any additional materials needed and are readily available before using the Cyclone kit.
- Organize all required components before starting any procedure.
- Follow all required safety instructions for safe handling of samples and reagents.

Preparation of Reagents and Buffers

- Ensure all working solutions are prepared according to the recommendations included in the protocol.
- All working solutions should be marked with the date of preparation to ensure those reagents are properly maintained.
- Evaluate each of the working solutions prior to each use to ensure there are no precipitates present. If salts or precipitates formed, they must be re-dissolved before use by warming the solution to 37ºC followed by equilibrating to room temperature. Gently invert to mix.
- ALWAYS use appropriate controls when performing any procedure.

Prepare Wash Buffers

Prepare each wash buffer according to the table below by adding the indicated volume of molecular grade ethanol (96-100%) to each concentrated wash solution before the first use. Gently invert to mix.

Material Name	Wash Buffer 1	Wash Buffer 2
Concentrated Wash Buffer	25 mL	11 mL
Molecular Grade Ethanol (96-100%)	15 mL	44 mL
Final Volume	40 mL	55 mL

Prepare Carrier RNA

Carrier RNA is provided as a dried material in an aluminum pouch to prevent light and moisture exposure.

- Reconstitute carrier RNA by adding 300 µL of Eluent to the vial.
- Incubate the freshly reconstituted carrier RNA for 5 minutes at room temperature.
- Mix the vial thoroughly using pulse vortex or inversion, then briefly centrifuge the vial.
- Use reconstituted carrier RNA immediately or store at – 20ºC.
- Do not freeze/thaw carrier RNA >10 times.
- Reconstituted carrier RNA may be aliquoted prior to use or stored to avoid unnecessary freeze/thaw cycles.

Calculating Required Carrier RNA

When beginning a new procedure, calculate the volume of carrier RNA required to prepare the working Lysis Solution using the following formula:

- N x 0.22 mL = Y mL
- Y mL x 25.0 µL/mL = Z µL

Where N is the number of samples to be processed, Y is the calculated volume (mL) of Lysis Solution, and Z is the volume (µL) of carrier RNA to add to Y mL of Lysis Solution.

Example: Prepare carrier RNA and Lysis Solution for 5 samples (N=5).

5 x 0.22 mL = 1.1 mL
1.1 mL x 25.0 µL/mL = 27.5 µL
Pipette 27.5 µL carrier RNA into 1.1 mL of Lysis Solution

Add the calculated volume of reconstituted carrier RNA to the Lysis solution and pulse vortex to mix.

Purification of Nucleic Acids Protocol

Prepare Spin Column

1. Before starting the procedure, each new Spin Column must be prepared by treating it with Column Preparation Liquid. Column treatment maximizes binding of the nucleic acids to the membrane, resulting in more consistent yields.

IMPORTANT! Close the bag with Spin Columns tightly after each use.

2. Add 50 µL of Column Preparation Liquid to the center of Spin Column membrane, so that the membrane is entirely moistened.

IMPORTANT! Do not centrifuge the prepared column. The prepared column should be stored at room temperature until it is used for sample processing.

Lyse Sample

IMPORTANT! Prepare Lysis Solution with Carrier RNA prior to use. Do not add Proteinase K directly to working Lysis Solution.

1. Combine 200 µL of sample, 200 µL of working Lysis Solution, and 50 µL of proteinase K to an empty 1.5-mL lysis tube. Mix thoroughly by pipetting or close cap and pulse-vortex.
2. Incubate the sample for 15 minutes at room temperature.
3. Centrifuge for 3–5 seconds at full speed to collect any sample solution from the inside of the lid.

Bind Sample

1. Add 300 µL of ethanol (96-100%) and mix by pipetting or close cap and pulse-vortex.
2. Incubate the sample at room temperature for 3 minutes.
3. Centrifuge for 3–5 seconds at full speed to collect drops from the inside of the lid.

Wash Sample

1. Transfer 750 µL of lysate to the prepared Spin Column preassembled within the wash tube.
2. Centrifuge the column for 1 minute at 8,000 rpm.
3. Discard the Wash Tube containing flow-through.
4. Transfer the Spin Column into a new 2.0-mL Wash Tube.
5. Add 700 µL of **Wash Buffer 1** to the Spin Column.
6. Centrifuge the column for 1 minute at 8,000 rpm.
7. Discard the Wash Tube containing flow-through.
8. Transfer the Spin Column into a new 2.0-mL Wash Tube.
9. Add 500 µL of **Wash Buffer 2** to the Spin Column.
10. Centrifuge the column for 1 minute at 8,000 rpm.
11. Discard the Wash Tube containing flow-through.
12. Transfer the Spin Column into a new 2.0-mL Wash Tube.
13. Centrifuge the column for 3 minutes at 8,000 rpm.
14. Discard the Wash Tube containing flow-through.
15. Transfer the Spin Column into a new 2.0-mL capped collection tube.

Elute Sample

1. Add 100 µL of Eluent or Molecular Grade Water to the center of Spin Column membrane. [*Note: Elution volumes may range 50 - 150 µL*]
2. Incubate for 3 minutes at room temperature.
3. Centrifuge the column for 1 minute at 8,000 rpm.
4. Discard the Spin Column.
5. Elution tube contains purified sample nucleic acids. Use the purified nucleic acids immediately or store any remaining sample refrigerated (2–8°C) up to 96 hours. For storage of extracted nucleic acids greater than 96 hours, it is recommended that samples should be stored frozen (-20°C or colder) in a non-frost-free freezer.

Optional Pre-treatment Steps for Purification of Nucleic Acids

Nucleic Acid Purification from Buccal Swabs

1. Collect buccal swab sample according to the approved procedure.
2. Place buccal swab into 200 µL of 1x PBS and swirl to integrate.
3. Proceed to **Purification of Nucleic Acids Protocol**

Nucleic Acid Purification from Complex Urine Samples

1. Add 0.5 mL of 0.5 M EDTA to 4.5 mL of urine. Gently invert to mix.
2. Centrifuge 10 minutes at 3,500 rpm.
3. Discard the supernatant.
4. Resuspend the pellet in 200 µL of 1x PBS.
5. Proceed to **Purification of Nucleic Acids Protocol**

Nucleic Acid Purification from Saliva Samples

1. Centrifuge saliva sample for 5 minutes at 3,500 rpm.
2. Resuspend cells in 200 µL of 1x PBS.
3. Proceed to **Purification of Nucleic Acids Protocol**

Technical Support

Contact DTPM Technical Support for any questions regarding use or for assistance with troubleshooting.

You may reach Technical Support using the following contacts:

Email: **HELP@DTPM.com**
Phone: (256) 845-1261