

# FFPE RNA/DNA Purification Plus Kit Dx



CE



# **Product Insert**



# Intended Use

Norgen's FFPE RNA/DNA Purification Plus Kit Dx provides a rapid method for the sequential isolation and purification of total RNA (including microRNA) and genomic DNA from formalin-fixed paraffin-embedded (FFPE) tissue samples for subsequent *in vitro* diagnostic use. Using formalin to fix tissues leads to crosslinking of the nucleic acids and proteins, and the process of embedding the tissue samples can also lead to fragmentation of the nucleic acids over time. Norgen's FFPE RNA/DNA Purification Plus Kit Dx provides conditions that allow for the partial reversing of the formalin modifications, resulting in a high quality and yield of nucleic acids. The kit is able to purify all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA), depending on the age of the FFPE tissue as the degree of fragmentation of the RNA will increase over time.

This kit is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of nucleic acids followed by signal detection or amplification. Any diagnostic results generated using the RNA and DNA isolated with Norgen's FFPE RNA/DNA Purification Plus Kit Dx in conjunction with an *in vitro* diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, suitable controls for downstream applications should be used.

Norgen's FFPE RNA/DNA Purification Plus Kit Dx is intended for use by professional users such as technicians, physicians and biologists experienced and trained in molecular biological techniques.

Norgen's FFPE RNA/DNA Purification Plus Kit Dx does not provide a diagnostic result. It is the sole responsibility of the user to use and validate the kit in conjunction with a downstream *in vitro* diagnostic assay.

Component	Product # Dx54300 (50 preps)
Digestion Buffer	2 x 20 mL
Binding Solution	2 x 20 mL
Enzyme Incubation Buffer	6 mL
RNA Wash Solution	24 mL
DNA Wash Solution	38 mL
RNA Elution Solution	6 mL
DNA Elution Buffer	15 mL
Proteinase K	2 x 12 mg
DNase I	200 μL
RNA Purification Micro Columns	50
DNA Purification Micro Columns	50
Collection Tubes	100
Elution tubes (1.7 mL)	100
Product Insert	1

#### **Kit Components**

# Label Legend

(	Σ	LOT	REF	Σ	***	IVD	i	
Do not reuse	Use by	Batch Code	Catalogue Number	Contains sufficient for <n> tests</n>	Manu- facturer	In Vitro Diagnostic Medical Device	Consult instructions for use	Temper- ature limitation

#### Advantages

- CE-IVD marked in accordance with EU Directive 98/79/EC
- Fits into in vitro diagnostic workflows
- Separate fraction of RNA and DNA
- High yields and quality of nucleic acids
- Isolate total RNA, from large rRNA down to microRNA (miRNA)
- No phenol or chloroform extractions

#### Specifications

Kit Specifications			
Column Binding Capacity (RNA)	35 μg		
Column Binding Capacity (gDNA)	10 μg		
Maximum Column Loading Volume	600 μL		
Size of RNA Purified	All sizes, including small RNA (<200 nt)		
Maximum Amount of Starting Material	4 sections $\leq 20 \ \mu M$ thick 10 mg of unsectioned block		

# **Storage Conditions and Product Stability**

The DNAse I and Proteinase K should be stored at -20°C upon arrival. All other solutions and kit components should be kept tightly sealed and stored at room temperature. All solutions and plastics can be used until the expiration date specified on their labels.

#### Precautions

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

The **Binding Solution** contains guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions

#### **Customer-Supplied Reagents and Equipment**

You must have the following in order to use the FFPE RNA/DNA Purification Plus Kit Dx:

- Benchtop microcentrifuge
- 50 55°C Incubator or Water Bath
- 80°C Incubator or Water Bath
- 90°C Incubator or Water Bath
- 96 100% ethanol
- Xylene, histological grade
- Ice Bucket

# **Procedures**

All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5}) (r)}}$$

where RCF = required gravitational acceleration (relative centrifugal force in units of g); r = radius of the rotor in cm; and RPM = the number of revolutions per minute required to achieve the necessary *g*-force.

# Working with RNA

RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense against these enzymes.

- The RNA area should be located away from microbiological work stations
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination
- There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only
- All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water
- Clean all surfaces with commercially available RNase decontamination solutions
- When working with purified RNA samples, ensure that they remain on ice during downstream applications

# Notes Prior to Use

- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~ 14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use.
- All enzymes provided should remain at the storage temperature indicated on each vial until use.
- Reconstitute each vial of the **Proteinase K** in 600 μL of molecular biology grade water, aliquot into small fractions and store the unused portions at -20°C until needed.
- Prepare a working concentration of the **RNA Wash Solution** by adding 66 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated **RNA Wash Solution**. This will give a final volume of 90 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Prepare a working concentration of the **DNA Wash Solution** by adding 90 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated **DNA Wash Solution**. This will give a final volume of 128 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Optional: For sensitive downstream applications or target transcripts of low quantity, add 10 μL of β-mercaptoethanol (provided by the user) to each 1 mL of Binding Solution required. β-mercaptoethanol is toxic and should be dispensed in a fume hood. Alternatively, Binding Solution can be used as provided.
- The maximum recommended input is four sections of  $\leq$  20  $\mu$ m thick. Alternatively, an unsectioned block of up to 10 mg may be used.

- It is important to obtain sections from the interior of an FFPE block in order to minimize RNA or DNA damage by oxidation.
- It is important to work quickly during this procedure.
- This kit is provided with 2 separate columns. When columns are removed from the labelled bags they are supplied in they can easily be identified as follows:
  - DNA Purification Micro Columns column has blue and white contents
  - o RNA Purification Micro Columns column has grey and white contents





# Section 1: Deparaffinization of FFPE Samples

### 1. Deparaffinization

a. Cut four sections up to 20  $\mu$ m thick from the interior of an FFPE tissue block using a microtome. Trim off any excess paraffin.

**Note**: Alternatively, from an FFPE block, cut out up to 10 mg of unsectioned core. Trim off any excess paraffin. Grind the sample into fine powder using liquid nitrogen.

- b. Transfer the sections or ground block into an RNase-free microcentrifuge tube.
- c. Add 1 mL of xylene to the sample. Mix by vortexing.
- d. Incubate at 50°C for 5 minutes.
- e. Centrifuge the sample at 14,000 x g (~ 14,000 RPM) for 2 minutes.
- f. Carefully remove the xylene without dislodging the pellet.
- g. Add 1 mL of 96 100 % ethanol. Mix by vortexing.
- h. Centrifuge the sample at 14,000 x g (~ 14,000 RPM) for 2 minutes.
- i. Carefully remove the ethanol without dislodging the pellet.
- j. Repeat Step **1g** to Step **1i** for a second time.
- k. Air dry the pellet for about 10 minutes at room temperature.

Note: It is important to remove the ethanol completely.

I. Proceed to Section 2. Total RNA Purification

# Section 2: Total RNA Purification

# 2. Lysate Preparation

- a. Add 300  $\mu$ L of **Digestion Buffer** and 10  $\mu$ L of the reconstituted **Proteinase K** to the sample. Mix by vortexing.
- b. Incubate at 55°C for 15 minute. Vortex to mix occasionally.
- c. Allow the sample to cool down by placing the tube containing the sample on ice for 3 minutes.
- d. Centrifuge the sample at 14,000 x g (~ 14,000 RPM) for 3 minutes.
- e. Carefully transfer the RNA-containing supernatant to a new RNase-free microcentrifuge tube. **Retain the microcentrifuge tube containing the pellet for DNA Purification.**

**Note:** The DNA-containing pellet can be stored for 2 hours at room temperature, for up to 24 hours at 2–8°C, or at –20°C for extensive storage.

f. Incubate the tube of the RNA-containing lysate at 80°C for 15 minutes. Vortex to mix occasionally.

Note: Do not exceed 15 minutes of incubation at 80°C as this will increase RNA fragmentation.

- g. Add 300 µL of **Binding Solution**. Vortex to mix.
- h. Add 600  $\mu$ L of 96 100 % ethanol. Vortex to mix.

#### 3. Binding RNA to Column

- a. Assemble an RNA Purification Micro Column with one of the provided collection tubes
- b. Apply up to 600 µL of the lysate with the ethanol (from **Step 2h**) onto the column and centrifuge for 1 minute at ≥ 3,500 x g (~6,000 RPM).

- **Note:** Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute at **14,000 x** g (~14,000 RPM).
- c. Discard the flowthrough. Reassemble the spin column with its collection tube.
- d. Repeat Step **3b** and **3c** until all lysate has passed through the column.

#### **Optional Step:**

Norgen's FFPE RNA/DNA Purification Plus Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional **On-Column DNA Removal Protocol** is provided in Appendix A for maximum removal of residual DNA that may affect sensitive downstream applications. This step should be performed at this point in the protocol using the provided DNase I.

#### 4. RNA Column Wash

a. Apply 500  $\mu$ L of **RNA Wash Solution** to the column and centrifuge for 1 minute at 14,000 x g (~ 14,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.

**Note:** Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

- Apply 500 μL of **RNA Wash Solution** to the column and centrifuge for 1 minute at 14,000 x g (~ 14,000 RPM).
- c. Discard the flowthrough and reassemble the spin column with its collection tube
- d. Wash column a third time by adding another 500  $\mu$ L of **RNA Wash Solution** and centrifuging for 1 minute at 14,000 x g (~ 14,000 RPM).
- e. Discard the flowthrough and reassemble the spin column with its collection tube.
- f. Spin the column for 2 minutes 14,000 x g (~ 14,000 RPM) in order to thoroughly dry the resin. Discard the collection tube.

# 5. RNA Elution

- a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
- b. Add 20 50  $\mu$ L of **RNA Elution Solution** to the column.
- c. Centrifuge for 2 minutes at 200 x g (~2,000 RPM), followed by a 1 minute spin at 14,000 x g (~14,000 RPM). Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at 14,000 x g (~14,000 RPM) for 1 additional minute.
  - **Note:** As little as 15  $\mu$ L of **RNA Elution Solution** could be used for higher RNA concentration. However, the RNA yield may be reduced when a smaller elution volume is used. For maximum RNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 5b** and **5c**).

# 6. Storage of RNA

The purified RNA may be stored at  $-20^{\circ}$ C for a few days. It is recommended that samples be placed at  $-70^{\circ}$ C for long term storage.

# Section 3: Total DNA Purification

# 7. Lysate Preparation

- a. Add 300  $\mu$ L of **Digestion Buffer** and 10  $\mu$ L of the reconstituted **Proteinase K** to the DNA-containing pellet obtained from Step **2e**. Mix by vortexing.
- b. Incubate at 55°C for 1 hour. Vortex to mix occasionally.
- c. Incubate at 90°C for 2 hour. Vortex gently occasionally to mix.
- d. Allow the sample to cool down by placing the tube containing the sample on ice for 3 minutes.

**Note:** Norgen's FFPE RNA/DNA Purification Plus Kit isolates DNA with minimal amounts of RNA contamination. However, if it is desirable to remove any trace amount of RNA, add 4  $\mu$ L of RNase A (10 mg/mL) to the cooled lysate and incubate at room temperature for 5 minutes.

- e. Add 300  $\mu$ L of **Binding Solution**. Vortex to mix.
- f. Add 250  $\mu$ L of 96 100 % ethanol. Vortex to mix.

# 8. Binding DNA to Column

- a. Assemble an DNA Purification Micro Column with one of the provided collection tubes.
- b. Apply up to 600  $\mu$ L of the lysate with the ethanol (from **Step 7f**) onto the column and centrifuge at 14,000 x g (~ 14,000 RPM) for 1 minute.
- c. Discard the flowthrough. Reassemble the spin column with its collection tube.
- d. Repeat Step 8b and 8c until all lysate has passed through the column.

#### 9. DNA Column Wash

a. Apply 600  $\mu$ L of **DNA Wash Solution** to the column and centrifuge for 1 minute at 14,000 x g (~ 14,000 RPM).

**Note:** Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

- b. Discard the flowthrough and reassemble the spin column with its collection tube. Apply 600  $\mu$ L of **DNA Wash Solution** to the column and centrifuge for 1 minute at 14,000 x g (~ 14,000 RPM).
- c. Discard the flowthrough and reassemble the spin column with its collection tube
- d. Wash column a third time by adding another 600  $\mu$ L of **DNA Wash Solution** and centrifuging for 1 minute at 14,000 x g (~ 14,000 RPM).
- e. Discard the flowthrough and reassemble the spin column with its collection tube.
- f. Spin the column for 2 minutes 14,000 x g (~ 14,000 RPM) in order to thoroughly dry the resin. Discard the collection tube.

# 10. DNA Elution

- a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
- b. Add 20 50  $\mu$ L of **DNA Elution Buffer** to the column. Incubate the assembly at room temperature for 1 minute.
- c. Centrifuge 1 minute at 14,000 x g (~14,000 RPM). Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at 14,000 x g (~14,000 RPM) for 1 additional minute.
  - **Note:** For maximum DNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 10b** and **10c**).

# 11. Storage of DNA

The purified DNA may be stored at  $4^{\circ}$ C for a few days. It is recommended that samples be placed at -20 or  $-70^{\circ}$ C for long term storage.

# **Appendix A**

# Protocol for Optional On-Column DNA Removal

#### Notes Prior to Use

- This optional step is carried out if genomic DNA-free RNA is required.
- Prepare a DNase I mixture by adding 4 μL of the provided RNase-free DNase I to 96 μL of **Enzyme Incubation Buffer** for each isolation.
- a. Apply 500 μL of **RNA Wash Solution** to the column and centrifuge at 14,000 x g (~14,000 RPM) for 2 minutes. Discard the flowthrough.
  - **Note:** Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.
- b. Apply 100 μL **Enzyme Incubation Buffer** mix containing the RNase-free DNase I to the column and centrifuge at 14,000 x g (~14,000 RPM) for 1 minute.

**Note:** Ensure that the entire volume of DNase I mix passes through the column. If needed, spin at 14,000 x g for an additional minute.

c. After the centrifugation in Step b, pipette the flowthrough that is present in the collection tube back onto the top of the column.

**Note:** Ensure that Step c is performed in order to ensure maximum DNase activity and to obtain maximum yields of RNA, in particular for small RNA species.

- d. Incubate at room temperature for 15 minutes.
- e. Proceed to Step 4b without further centrifugation.

Note: Add the RNA Wash Solution directly to the column containing the Enzyme Incubation Buffer mix containing the RNase-free DNase I.

#### **Technical Support**

Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at <u>techsupport@norgenbiotek.com</u>.

# Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA or DNA Recovery	Incomplete lysis of cells or tissue	Ensure that the appropriate amount of <b>Digestion Buffer</b> and Proteinase K was used. Increase the incubation time.
	Column has become clogged	Do not exceed the recommended amounts of starting materials. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels. See also "Clogged Column" below.
	An alternative elution solution was used	It is recommended that the <b>RNA Elution Solution</b> and <b>DNA Elution Buffer</b> supplied with this kit be used for maximum RNA or DNA recovery.
	Ethanol or Binding Buffer was not added to the lysate	Ensure that the appropriate amount of ethanol and Binding Buffer is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solutions	Ensure that 66 mL of 96 - 100% ethanol is added to the supplied <b>RNA Wash Solution</b> prior to use and 90 mL of 96-100% ethanol is added to the supplied <b>DNA Wash Solution</b> prior to use.
	Low nucleic acid content in cells or tissues used	Different tissues and cells have different nucleic acid contents, and thus the expected yield of nucleic acid will vary greatly from these different sources. Please check literature to determine the expected nucleic acid content of your starting material.
Clogged Column	Insufficient solubilization of cells or tissues	Ensure that the appropriate amount of <b>Digestion Buffer</b> with Proteinase K added was used. Increase the incubation time.
	Maximum number of sections or amount of tissue exceeds kit specifications	Refer to specifications to determine if amount of starting material falls within kit specifications.
	Clarified lysate was not used for the binding step	Ensure that after the lysis step, the sample is centrifuged if significant precipitates are present, and that only the clarified lysate is used in subsequent steps.
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 15°C may cause precipitates to form that can cause the columns to clog.

Problem	Possible Cause	Solution and Explanation
RNA is Degraded	FFPE sample is old	The quality of RNA purified may drastically decrease in old samples. For best performance, freshly prepared samples are highly recommended.
	RNase contamination	RNases may be introduced during the use of the kit. Ensure proper procedures are followed when working with RNA. Please refer to " <i>Working with RNA</i> " at the beginning of this user guide.
	Too long incubation of lysate at high temperature	In order to maintain the integrity of the RNA, it is important that the procedure be performed according to the time indicated. This is especially important for the lysate preparation step when the sample is incubated at 55 and 80°C for 15 minutes each. Incubation beyond the time indicated may lead to fragmentation of RNA.
	Improper storage of the purified RNA	For short term storage RNA samples may be stored at $-20^{\circ}$ C for a few days. It is recommended that samples be stored at $-70^{\circ}$ C for longer term storage.
	Prolonged incubation at 55°C	In order to reverse formalin crosslinks, an incubation at 55°C is required which may lead to degraded RNA.
Nucleic acids does not perform well in downstream applications	Nucleic acids were not washed 3 times with the provided Wash Solution	Traces of salt from the binding step may remain in the sample if the column is not washed 3 times with Wash Solution. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
	Formalin crosslink was not completely reversed	Ensure the sufficient incubation at 80 or 90°C is performed to remove formalin crosslink.
Genomic DNA contamination	Large amounts of starting material used	Perform RNase-free DNase I digestion on the RNA sample after elution to remove genomic DNA contamination.

#### **Product Use Restriction**

Norgen's FFPE RNA/DNA Purification Plus Kit Dx provides a rapid method for the isolation and purification of total RNA (including microRNA) from formalin-fixed paraffin-embedded (FFPE) tissue samples subsequent *in vitro* diagnostic use.

This kit is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of RNA followed by signal detection or amplification. Any diagnostic results generated using the RNA isolated with Norgen's FFPE RNA/DNA Purification Plus Kit Dx in conjunction with an *in vitro* diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, suitable controls for downstream applications should be used.

Norgen's FFPE RNA/DNA Purification Plus Kit Dx is intended for use by professional users such as technicians, physicians and biologists experienced and trained in molecular biological techniques.

Norgen's FFPE RNA/DNA Purification Plus Kit Dx does not provide a diagnostic result. It is the sole responsibility of the user to use and validate the kit in conjunction with a downstream *in vitro* diagnostic assay.

The respective user is liable for any and all damages resulting from application of Norgen's FFPE RNA/DNA Purification Plus Kit Dx for use deviating from the intended use as specified in the user manual.

All products sold by Norgen Biotek are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately. The kit contents are for laboratory use only, and they must be stored in the laboratory and must not be used for purposes other than intended. The kit contents are unfit for consumption.

#### Authorized Representative



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