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In Vitro Transcription Clean-Up and Concentration Micro Kit Product Insert
Product # 52900

Norgen's *In Vitro* Transcription Clean-Up and Concentration Micro Kit provides a rapid, simple and efficient procedure for the purification and concentration of up to 35 µg of RNA transcripts from *in vitro* transcription reactions. The kit purifies RNA transcripts from the various impurities used during the *in vitro* transcription reaction, including polymerases, unincorporated dNTPs, salts and DTT, without the use of phenol, chloroform or alcohol precipitation. The kit is robust with transcripts ≥ 100b and provides a high quality product with up to 90% recovery. The purified transcripts can be used in a number of downstream applications including Northern Blotting and Nuclease Protection Assays.

Norgen's Purification Technology
Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The RNA transcript is preferentially purified from other *in vitro* transcription reaction impurities such as proteins and salts without the use of phenol or chloroform. The process involves first mixing the *in vitro* transcription reaction sample with Binding Solution (please see the flow chart on page 3). Isopropanol is then added and the mixture is loaded onto a spin-column. Norgen's resin binds RNA in a manner that depends on ionic concentrations. Thus only the RNA will bind to the column, while the contaminating proteins or nucleotides will be removed in the flowthrough. The bound RNA is then washed three times with the provided Wash Solution in order to remove any remaining impurities. The purified RNA transcript is then eluted with Elution Solution. The purified RNA transcript is of the highest integrity, and can be used in a number of downstream applications.

This kit is designed to process 25 samples.

Specifications:

Kit Specifications	
Column Binding Capacity	35 µg
Maximum Column Loading Volume	600 µL
Size of RNA Purified	> 100b
Time to Complete 10 Purifications	15 minutes
Maximum Amount of Starting Material	35 µg of RNA
Minimum Elution Volume	15 µL

Advantages:

- Complete column purification - Transcripts are column cleaned and/or concentrated, eliminating hazardous and labour-intensive phenol-based procedures.
- Cleans *in vitro* transcription reaction mixtures - Efficient removal of buffers and enzymes in addition to dNTPs.
- Rapid procedure – Clean and concentrate 10 samples in 15 minutes.
- Small elution volume – Purified transcripts can be eluted in as low as 15 µL
- Provides high quality RNA transcripts - The purified RNA transcripts are of the highest quality and can be used in a number of downstream applications.

Kit Components:

Component	Contents
Binding Solution	15 mL
Wash Solution	8.5 mL
Elution Solution	3 mL
Micro Spin Columns	25
Collection Tubes	25
Elution tubes (1.7 mL)	25
Product Insert	1

Storage Conditions and Product Stability
All solutions should be kept tightly sealed and stored at room temperature (15-25°C) for up to 1 year without showing any reduction in performance.

Precautions and Disclaimers
This kit is designed for research purposes only. It is not intended for human or diagnostic use. 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*.

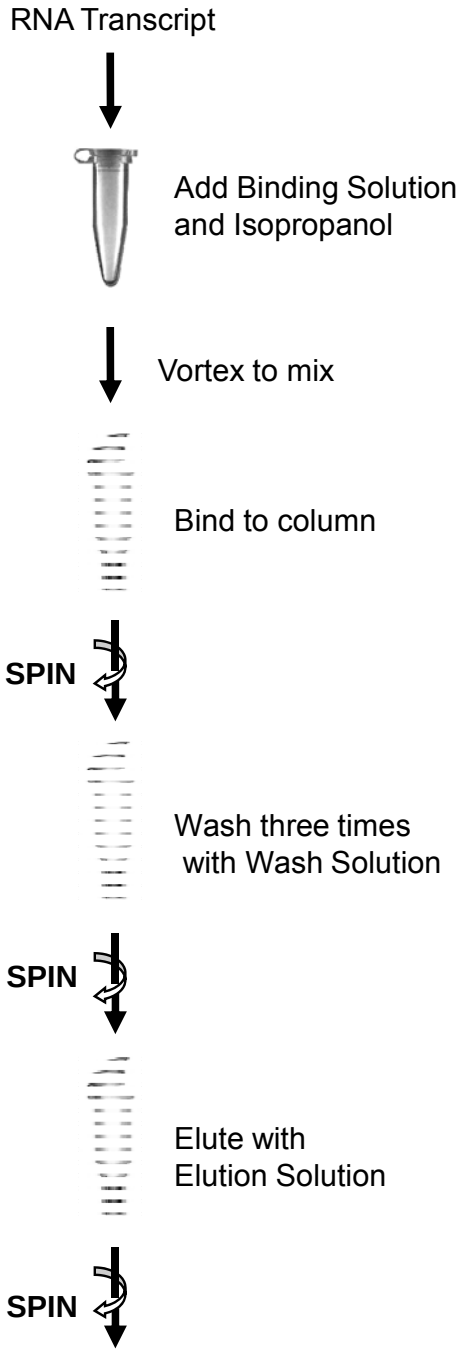
Customer-Supplied Reagents and Equipment
- Benchtop microcentrifuge
- Micropipettors
- Nuclease-free water
- β-mercaptoethanol
- Isopropanol
- 96 - 100% ethanol

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

Flow Chart

Procedure for RNA transcript purification using Norgen's *In Vitro* Transcription Clean-Up and Concentration Micro Kit



Purified RNA Transcript

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.

Notes Prior to Use

- 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.
- Prepare an appropriate amount of **Binding Solution** by adding 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.
- Prepare a working concentration of the **Wash Solution** by adding 32.5 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution**. This will give a final volume of 41 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- It is important to work quickly during this procedure.
- The maximum sample input volume that can be processed is 200 µL.
- Digestion of the DNA template used in the *in vitro* transcription reaction should be performed before cleaning the RNA transcript. Ensure that RNase-free DNase is used for the digestion step.
- The procedure below outlines the clean-up and concentration of 100 µL and 200 µL of *in vitro* transcription reaction. The volumes of solutions to be used for 100 µL are stated first, and the volumes of solutions for be used for 200 µL are shown in brackets.

1. Sample Preparation
The protocol can be used to process 100 µL (200 µL) of *in vitro* starting transcription reaction volume.

a. Adjust the volume of the sample to 100 µL (200 µL) by adding nuclease-free water.
b. Add 250 µL (500 µL) of **Binding Solution** to the sample. Mix by vortexing for 10 seconds.
c. Add 250 µL (500 µL) of isopropanol. Mix by vortexing for 10 seconds.

2. Binding to Column

a. Assemble a column with one of the provided collection tubes.
b. Apply up to 600 µL of the sample with the isopropanol (from **Step 1c**) onto the column and centrifuge for 1 minute at **14,000 x *g* (~14,000 RPM)**.
c. Discard the flowthrough. Reassemble the spin column with its collection tube.
d. Repeat steps 2b and 2c as required to bind the entire sample to the column.

3. Column Wash

a. Apply 400 µL of **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.
c. Repeat steps **3a** and **3b** to wash the column a second time.
d. Wash column a third time by adding another 400 µL of **Wash Solution** and centrifuging for 1 minute.
e. Discard the flowthrough and reassemble the spin column with its collection tube.
f. Spin the column for 2 minutes at **14,000 x *g* (~14,000 RPM)** in order to thoroughly dry the resin. Discard the collection tube.

4. Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 50 µL of **Elution Solution** to the column.

Note: For higher concentrations, a lower elution volume may be used. A minimum volume of 15 µL is recommended.

c. Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by 2 minutes 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.

5. Storage of Purified RNA transcripts

The purified RNA transcript samples should be stored at –70°C.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	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 RNA Elution Solution supplied with this kit be used for maximum RNA recovery.
	Isopropanol was not added to the lysate	Ensure that the appropriate amount of isopropanol is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solution	Ensure that the correct volume of 96-100% ethanol is added to the supplied Wash Solution prior to use.
Clogged Column	High amounts of RNA in the input	Ensure that no more than 35 µg of RNA is used as the input for each column.
	High amounts of DNA present in sample	Do not exceed the amount of DNA recommended by the manufacturer to be used in the *in vitro* transcription reaction. DNase treatment with RNase-free DNase can be used before cleaning the sample to remove the DNA template.
	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	RNase contamination	RNases may be introduced during the use of the kit. Ensure proper procedures are followed when working with RNA. Please refer to "Working with RNA" at the beginning of this user guide.
	Procedure not performed quickly enough	In order to maintain the integrity of the RNA, it is important that the procedure be performed quickly
	Improper storage of the purified RNA	For short term storage RNA samples may be stored at –20°C for a few days. It is recommended that samples be stored at –70°C for longer term storage.
RNA does not perform well in downstream applications	RNA was not washed three times with the provided Wash Solution	Traces of salt from the binding step may remain in the sample if the column is not washed three times with the 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.
DNA contamination	Carryover of DNA template used in the *in vitro* transcription reaction	Do not exceed the amount of DNA recommended by the manufacturer to be used in the *in vitro* transcription reaction. DNase treatment with RNase-free DNase can be used before cleaning the sample to remove the DNA template.

Related Products	Product #
RNA Clean-Up and Concentration Kit	23600
PCR Purification Kit	14400
CleanAll DNA/RNA Clean-Up and Concentration Kit	23800
MiniSizer 50 bp DNA Ladder	11200

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 techsupport@norgenbiotek.com.

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