GENEMED SYNTHESIS, INC.
6203 Wood Lake Center Dr., Bldg. 2, San Antonio, TX 78244, USA
Toll free (800) 344-5337; **Phone**: (210) 745-5988; **Fax** (210) 745-5992
Email: info@genemedsyn.com, **Website**: www.genemedsyn.com

INSTRUCTION MANUAL

RNA Clean & Concentrator™-5

Catalog Nos. **ZR1015 & ZR1016**

Highlights

- Quick, 5 minute recovery of ultra pure RNA (≥17 nt) from enzymatic reactions and other samples.
- High quality RNA eluted in ≥6 µl is ready for library prep, reverse transcription, microarray, sequencing, etc.

Contents

Satisfaction of all GSI products is guaranteed. If you are dissatisfied with this product please call 1-800-344-5337

Notes:

[1] Add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **RNA Wash Buffer** concentrate (ZR1015) or 96 ml 100% ethanol (104 ml of 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate (ZR1016) before use.

[2] Compatible with: TRIzol®, TRI Reagent®, RNAzol®, QIAzol®, TriPure™, TriSure™ and other *acid-guanidinium-phenol* reagents.

Product Contents

RNA Clean & Concentrator™-5 (Kit Size)	**ZR1015** (50 Preps.)	**ZZR1016** (200 Preps.)
RNA Binding Buffer	25 ml	100 ml
RNA Prep Buffer	25 ml	4x 25 ml
RNA Wash Buffer[1] (concentrate)	12 ml	2x 24 ml
DNase/RNase-Free Water	1 ml	4 ml
Spin™ IC Columns	50	200
Collection Tubes	50	200
Instruction Manual	1	1

Note - Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

Storage Temperature – Store all kit components (*i.e.,* buffers, columns) at room temperature.

Specifications

- **Sample Sources** – DNase I-treated RNA, *in vitro* transcription products, the aqueous phase following TRIzol®/chloroform or similar[2] extraction (Appendix B, page 4).
- **RNA Size Limits** – From 17 nt to ~23 kb.
- **RNA Purity** – High quality RNA (A_{260}/A_{280} >1.8, A_{260}/A_{230} >1.8) suitable for reverse transcription, microarray, sequencing, etc.
- **RNA Recovery** – Up to 10 µg RNA can be eluted into ≥6 µl RNase-free water allowing for a highly concentrated sample.
- **RNA Storage** – RNA is eluted with RNase-free water and can be stored at ≤-70 ºC. The addition of RNase inhibitors is optional but highly recommended for prolonged storage.
- **Equipment Needed** – Microcentrifuge.

Note - This product is for research use only and should only be used by trained professionals. It is not intended for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.
TRI Reagent®, TRIzol® and RNAzol® (Molecular Research Center, Inc.), QIAzol® (Qiagen GmbH), TriPure™ (Roche, Inc.), TriSure™ (Bioline Ltd.), RNA*later*® (Ambion, Inc.).

Product Description

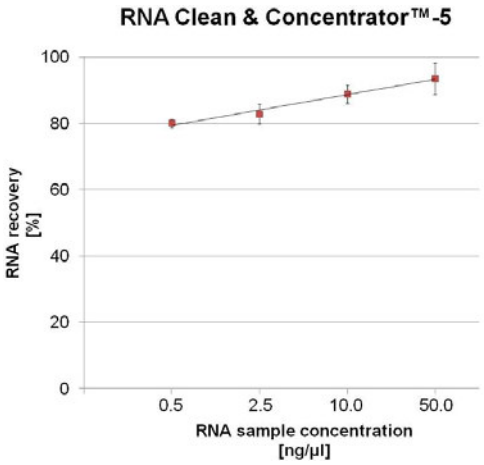
RNA Clean & Concentrator™-5 provides a simple and reliable method for the rapid preparation of up to 10 µg of high-quality RT-PCR-ready RNA. This simple procedure is based on the use of a unique single-buffer system and *Clean-Spin* column technology for selective recovery of total RNA (> 17 nt), large RNAs (> 200 nt), and/or small RNAs (17-200 nt) from almost any enzymatic reaction or liquid sample.

The procedure is easy! Add binding buffer and ethanol to your sample and then bind, wash and elute ultra pure RNA. The RNA can be eluted from the **Spin™ IC Column** in as little as 6 µl of RNase-free water. The highly-concentrated, purified RNA is suitable for all subsequent analyses and molecular manipulations.

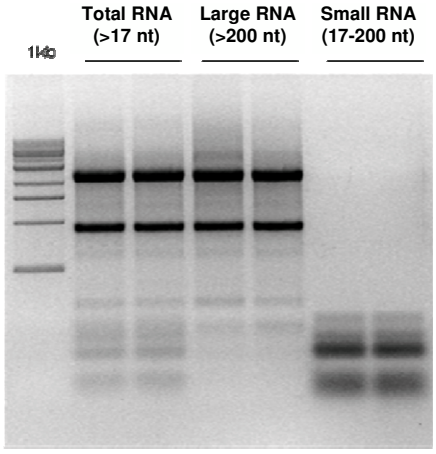
The entire procedure typically takes about 5 minutes.

Note:

For purification of DNA see the **DNA Clean & Concentrator™-5** and **-25** (Catalog Nos. ZD4013, ZD4014, ZD4033, ZD4034).



Concentration of diluted RNA samples. RNA was eluted with 20 µl RNase-free water (n = 3, total input = 1 µg RNA).



Purification of small and large RNAs into separate fractions. RNA Clean & Concentrator™ allows for purification of total RNA (> 17 nt), large RNAs (> 200 nt), and/or small RNAs (17-200 nt). See Appendix C.

Ensure RNA isolation is performed in an RNase-free environment.

Buffer Preparation

Before starting, add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **RNA Wash Buffer** concentrate (ZR1015) or 96 ml 100% ethanol (104 ml of 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate (ZR1016).

Protocol

Notes:

All centrifugation steps should be performed between 10,000 – 16,000 x *g*.
Following the below procedure, RNA species ≥17 nt will be recovered.

1. Add 2 volumes **RNA Binding Buffer** to each sample[1] and mix.

   Example: Mix 100 µl buffer and 50 µl sample.

2. Add an equal volume of ethanol (95-100%) and mix.

   Example: Add 150 µl ethanol to 150 µl sample/binding buffer.

3. Transfer the sample to the **Spin™ IC Column** in a **Collection Tube** and centrifuge for 30 seconds[2]. Discard the flow-through.
4. Add 400 µl **RNA Prep Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.
5. Add 700 µl **RNA Wash Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.
6. Add 400 µl **RNA Wash Buffer** to the column and centrifuge for 2 minutes to ensure complete removal of the wash buffer.
7. Place the column into an RNase-free tube (not provided). Add ≥6 µl **DNase/RNase-Free Water**[3] directly to the column matrix and centrifuge for 30 seconds.

   Eluted RNA can be used immediately or stored at -70°C.

[1] Adjust the sample volume to 50 µl (minimum).

Spin™ columns may be reloaded to process samples >800 µl.

[2] At this point, samples can be *in-column* DNase treated (Appendix A, page 4).

[3] For maximum recovery, increase the elution volume (≥ 15 µl) and/or repeat the elution.

Appendix A: *In-Column* DNase I Treatment

All centrifugation steps should be performed between 10,000 – 16,000 x *g*.

The DNase digestion procedure can be performed using the **DNase I Set** (E1009, not provided) or any other DNase I together with its dedicated reaction buffer. DNase I maintains activity in the **RNA Wash Buffer**.

1. Following RNA binding (page 3, step 3), wash the column with 400 µl **RNA Wash Buffer**. Centrifuge for 30 seconds. Discard the flow through.
2. For each sample to be treated, prepare a **DNase I reaction mix** in an RNase-free tube (not provided). Add the reagents in the following order:

DNase I	5 µl (1 U/µl)
10X Reaction Buffer	5 µl
RNA Wash Buffer (with ethanol added)	40 µl
	50 µl

3. Add 50 µl **DNase I reaction mix** directly to the column matrix. Incubate at room temperature (20-30ºC) for 15 minutes[1]. Then centrifuge for 30 seconds.
4. Continue with wash steps (page 3, step 4).

Appendix B: RNA Recovery from Aqueous Phase following Trizol® Extraction

1. Following Trizol®/chloroform or similar[2] extraction, carefully transfer the upper aqueous phase into an RNase-free tube (not provided).
2. For each volume of the aqueous phase (as measured or estimated), add 1 volume ethanol (95-100%) and mix.
3. Continue with purification (page 3, step 3).

Notes:

Unit definition – one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A_{260} units/min/ml of reaction mixture at 25°C.

[1] The optimal incubation time can vary.

[2] Compatible with: TRIzol®, TRI Reagent®, RNAzol®, QIAzol®, TriPure™, TriSure™ and other *acid-guanidinium-phenol* reagents.

Appendix C: Purification of Small and Large RNAs into Separate Fractions

All centrifugation steps should be performed between 10,000 – 16,000 x *g*.
This protocol requires two columns (per prep).

1. Mix an equal volume of **RNA Binding Buffer** and ethanol (95-100%).

   Example: Mix 50 µl buffer and 50 µl ethanol.

2. Add 2 volumes of the buffer/ethanol to the sample and mix.

   Example: Mix 100 µl buffer/ethanol and 50 µl sample.

3. Transfer the mixture to a **Spin™ Column** and centrifuge for 30 seconds. **Save the flow-through!**

Column: RNAs >200 nt	**Flow-through**: RNAs 17-200 nt
4. Continue to step 5.	Add 1 volume ethanol and mix. Example: Add 150 µl ethanol to 150 µl flow-through. Transfer the mixture to a **new column** and centrifuge for 30 seconds. Discard the flow-through.

5. Add 400 µl **RNA Prep Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.
6. Add 700 µl **RNA Wash Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.
7. Add 400 µl **RNA Wash Buffer** to the column and centrifuge for 2 minutes to ensure complete removal of the wash buffer.
8. Place the column into an RNase-free tube (not provided). Add ≥6 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge for 30 seconds.

   Eluted RNA can be used immediately or stored at -70°C