Automated Dynabeads™-Based RNA Purification with the KingFisher™ Apex Purification System

Catalog Numbers 65020D, 65021D, 65040D, 65042D, 65032D, 65030D, 49020D, 49021D, 49040D, 49041D

Pub. No. MAN1000199 Rev. A

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Invitrogen™ Dynabeads™ Carboxylic Acid for RNA Purification and the Dynabeads™ RNA Binding Buffer are designed for effective removal of NTPs, proteins, and other components of the in vitro transcription (IVT) reaction mix. The products are ideal for automated high-throughput screenings using liquid handlers or any of the KingFisher™ purification systems.

This guide describes automated RNA purification using Dynabeads™ Carboxylic Acid for RNA Purification and the KingFisher™ Apex Purification System. The instrument scripts that are provided are intended as a starting point for the RNA purification workflow using 100 µL of crude RNA input. Optimization based on template- and user-specific needs may be required.

This user guide is intended for experienced users of Dynabeads™ Carboxylic Acid for RNA Purification and Dynabeads™ RNA Binding Buffer. For detailed instructions, refer to the *Dynabeads™-Based Solid-Phase In Vitro Transcription and RNA Purification User Guide* (Pub. no. MAN0029518).

For information about available products or support documentation for GMP manufacturing of RNA vaccines and therapeutics, go to **thermofisher.com/dynabeadsmrna**. This guide can also be used with Cat. No. 49020D, 49021D, 49040D, and 49041D, which are available through OEM/commercial supply at **thermofisher.com/oem-commercial-supply**.

Technology overview

Dynabeads™ Carboxylic Acid for RNA Purification are monosized (1 µm) paramagnetic beads which are characterized by high speed to magnet. The bead concentration is 10 mg/mL, stored in purified water.

The product is used in combination with Dynabeads™ RNA Binding Buffer for purifying the crude RNA produced by the IVT reaction (see Figure 1). The RNA is mixed with the beads and the buffer is added. The RNA is thereby bound to the beads surface and remaining components of the reaction mixture are effectively removed by applying a magnet and discarding the supernatant in the following wash steps. The beads may be re-cycled at least six times. It is recommended to use the same volumes of buffer and the input RNA solution.

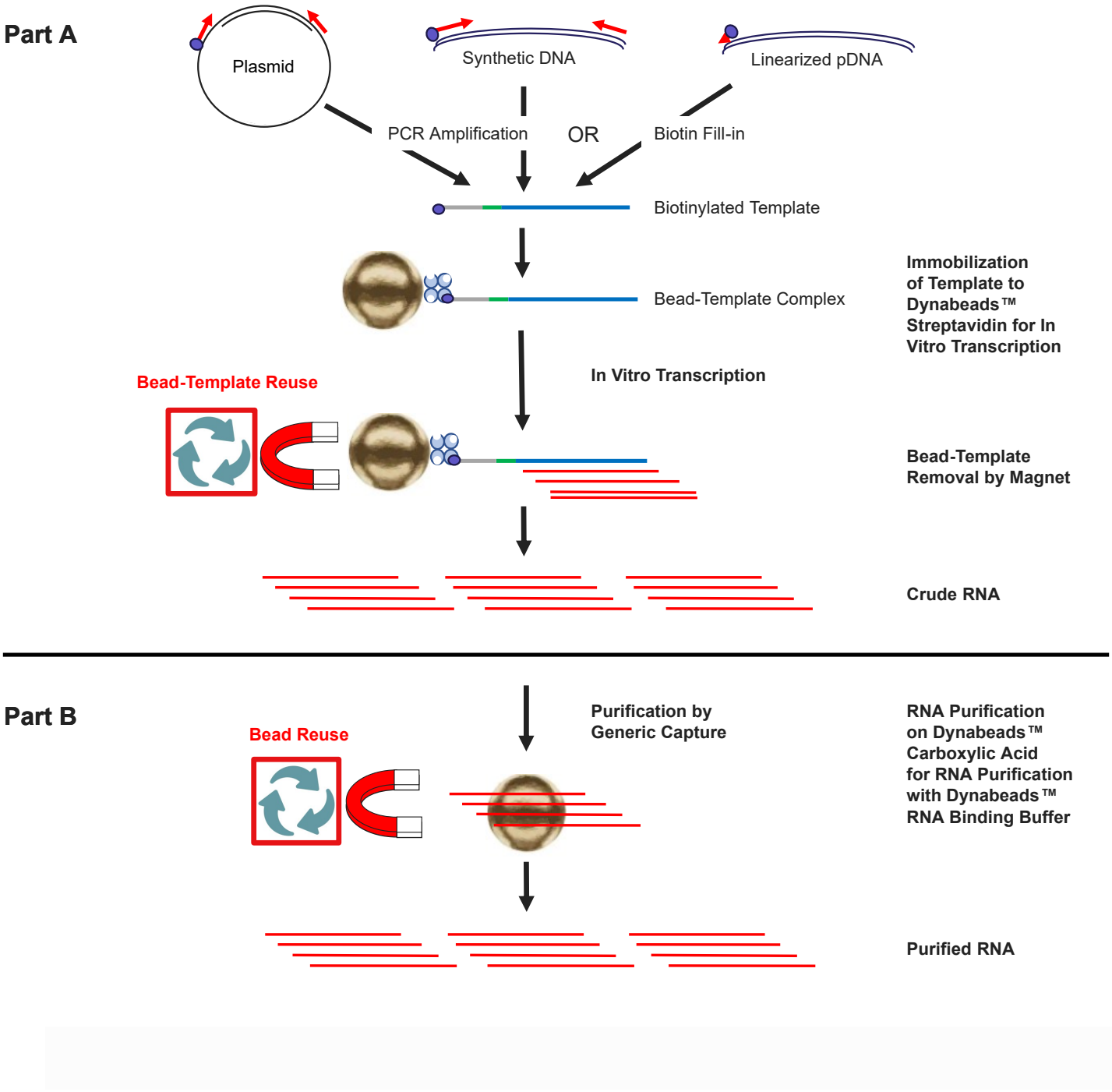

Figure 1 Workflow for RNA synthesis with Dynabeads™ Streptavidin for In Vitro Transcription (Part A) and RNA purification on Dynabeads™ Carboxylic Acid for RNA Purification (Part B). Beads in both parts may be re-cycled at least six times.

Contents and storage

Product	Cat. no.	Amount	Storage
Dynabeads™ Carboxylic Acid for RNA Purification[1]	65020D	2 mL	Store at 2–8°C.
	65021D and 49021D[2]	10 mL	
	49020D[2]	100 mL	
Dynabeads™ RNA Binding Buffer	65040D	20 mL	Store at 2–8°C. Protect from light exposure during storage.
	65042D and 49041D[2]	50 mL	
	49040D[2]	450 mL	

[1] Concentration is 10 mg/mL

[2] Only available through the Dynabeads OEM/Commercial Supply team. To review ordering information or to contact the Dynabeads™ OEM team, go to thermofisher.com/contact-dynabeads-plus.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Instrument	
KingFisher™ Apex with 96 Deep-Well Head	5400930
Reagents for RNA purification with the KingFisher™ Apex Purification System	
UltraPure™ DNase/RNase-Free Distilled Water	10977-049
70% ethanol[1]	MLS
Tris (1 M), pH 7.0, RNase-free	AM9850G
TE, pH 7.0, RNase-free[2]	AM9861
Consumables for KingFisher™ Apex with 96 Deep Well Head	
KingFisher™ Deep Well 96 Tip Comb, Barcoded	97002534B
KingFisher™ 96 Deep-Well Plate, Barcoded	95040460B

[1] Washing solution

[2] Example of elution buffer. A different RNase-free low salt buffer of choice can be used.

Workflow

The initial setup of the RNA purification protocol involves preparing the Dynabeads™ Carboxylic Acid for RNA Purification and preparing the crude RNA dilution if necessary. The KingFisher™ plate setup is then performed, with each plate filled with the required reagents in defined positions and correct volumes. The script is loaded onto the instrument and started, followed by plate loading and RNA purification processing.

The first step of the RNA purification involves prewashing the Dynabeads™ Carboxylic Acid for RNA Purification, followed by transferring the beads and mixed into the crude RNA. The instrument is then paused to allow for the addition of the Dynabeads™ RNA Binding Buffer. After pressing start, the script continues with RNA binding, washing, air drying, and finally elution of the purified RNA. For an experimental set-up as a suggested starting point, see Table 2.

For this RNA purification script, a total binding volume of 200 µL is used, consisting of 100 µL crude RNA and 100 µL Dynabeads™ RNA Binding Buffer. The efficiency of RNA capture on the bead surface depends not only on the right buffer concentration but also on RNA concentration and quantity of Dynabeads™ Carboxylic Acid for RNA Purification beads. Therefore, we recommend as maximum input approximately 5 µg/µL of 2500 nt RNA per 300 µg of beads.

Before you begin

Download the appropriate KingFisher™ Apex script from the Dynabeads™ Carboxylic Acid for RNA Purification or Dynabeads™ RNA Binding Buffer product page at www.thermofisher.com (search by catalog number), then install on the instrument. See Table 1.

Table 1 KingFisher™ Apex 96 DW Script

IVT volume	Script
100 µL	**KF_APEX_96DW_RNA_purification_200µLbindingvolume**

Prepare RNA samples, beads, and buffers

- Vortex the Dynabeads™ Carboxylic Acid for RNA Purification until fully homogenized. Ensure the beads stay fully mixed within the solution during pipetting.
- Prepare the RNA purification mix. If necessary, dilute the crude RNA to 5 µg/µL or lower in a total volume of 100 µL using RNase-free 10 mM Tris, pH 7. Place on ice.
- Prepare 70% ethanol.

Table 2 Experimental set-up

Reagent	Volume
Crude IVT mix [1]	100 µL[2]
Dynabeads™ Carboxylic Acid for RNA Purification	30 µL[3]
Dynabeads™ RNA Binding Buffer	100 µL
Elution output	100 µL

[1] Input RNA solution. It is recommended to use equal volumes of RNA Binding Buffer and the input RNA solution.
[2] For 2500 nt-long RNA we have tested concentrations up to 5 µg/µL with over 90% recovery. Recovery efficiency depends on RNA concentration and ratio of RNA molecules and beads.
[3] 300 µg

Prepare the processing plates

Prepare the processing plates according to the following table. Label the KingFisher™ Apex 96 DW plates with the plate name.

Table 3 Plate layout for KingFisher™ Apex 96 DW RNA Purification

Plate number	Plate ID	Plate type	Reagent	Volume per well
PL1	Tip comb	Place a KingFisher™ 96 tip comb for deep-well magnets in a KingFisher™ 96 Deep-Well Plate		
PL2	MyOne COOH	KingFisher™ 96 Deep-Well Plate	Dynabeads™ Carboxylic Acid for RNA Purification	30 µL
PL3	H_20		UltraPure™ DNase/RNase-Free Distilled Water	100 µL
PL4	Crude RNA[1]		Crude RNA	100 µL
PL5	Wash 1		70% ethanol	500 µL
PL6	Wash 2		70% ethanol	500 µL
PL7	Wash 3		70% ethanol	500 µL
PL8	Elution buffer		TE-buffer, pH 7	100 µL

[1] Dispense after pause Plate 4: Dynabeads™ RNA Binding Buffer

Process samples on the instrument

1. Label and fill the plates with the appropriate reagents and volumes, see Table 3.
2. Load script onto instrument. Start the script.
3. Load plates onto instrument as instructed, orienting A1 position on plate to A1 position on loading station.
4. After 3-4 minutes the instrumet will pause, dispense 100 µL of Dynabeads™ RNA Binding Buffer to the wells containing crude RNA in PL4.
5. Select ✓ on the instrument to continue with the RNA purification.

6. When script is completed, follow instructions for unloading plates.

7. Transfer purified RNA from PL8 to new RNase-free tubes for storage and analysis. Freeze the mRNA at -80°C.

8. For the quantification of the purified RNA, use the Qubit™ RNA BR Assay Kit (Cat. No. Q10210) or the NanoDrop™ One Microvolume UV-Vis Spectrophotometer.

Troubleshooting

Observation	Possible cause	Recommended action
Low RNA recovery	RNase-contaminated KingFisher™ plates.	Use sterile KingFisher™ plates.
	RNase-contaminated reagents.	Prepare RNase free buffers using RNase-free reagents.
	Wrong tip comb was used.	For the KingFisher™ Apex with 96 Deep Well Head use the KingFisher™ Deep Well 96 Tip Comb (Cat. No. 97002534). The tip comb should be V-shaped, and this shape is critical for proper mixing of the samples during the RNA purification processing.
	RNA precipitation.	A premix of crude RNA and Dynabeads™ RNA Binding Buffer before adding to Dynabeads™ Carboxylic Acid for RNA Purification can reduce RNA recovery, while RNA can be precipitated. Follow recommended protocol where the crude RNA is premixed with the beads before introduction of the RNA binding buffer.
Residual beads in plates	Bead collection time was too short.	Increase the collection bead time by increasing loop count and duration. The maximum for the KingFisher™ Apex instrument is 5 loops / 30 seconds.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN1000199 A

Revision	Date	Description
A	2 December 2024	New document for Automated Dynabeads™-Based RNA Purification with the KingFisher™ Apex Purification System.