Dynabeads™-Based Solid-Phase In Vitro Transcription and RNA Purification

Catalog Numbers 65005D, 65006D, 65020D, 65021D, 65040D, 65042D, 65030D, 65032D, 65034D, 65036D

Pub. No. MAN0029518 Rev. B

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

Invitrogen™ Dynabeads™ Streptavidin for In Vitro Transcription and Dynabeads™ Carboxylic Acid for RNA Purification beads are monosized (1 µm), paramagnetic beads that enable high-quality and scalable RNA production for various applications in basic research and drug discovery screening. The *in vitro* transcription (IVT) and purification procedures can be easily incorporated into manual workflows or automated using KingFisher™ instruments. Both workflows are designed to minimize handling time and reduce cost by enabling bead reuse (at least six times) in successive experiments.

Dynabeads™ Streptavidin for In Vitro Transcription beads are covalently coupled to streptavidin molecules to enable binding to a biotinylated DNA template. The DNA-bead complex can be used directly in IVT reactions without the need for any prior purification steps. Dynabeads™ Carboxylic Acid for RNA Purification beads are characterized by high speed-to-magnet and high pellet stability. The beads are designed for use in combination with Dynabeads™ RNA Binding Buffer to purify the crude RNA produced by solid-phase IVT reactions.

This guide describes IVT of a biotinylated DNA template that is immobilized on Dynabeads™ Streptavidin for In Vitro Transcription beads (Part A), followed by purification of the synthesized RNA using Dynabeads™ Carboxylic Acid for RNA Purification beads in combination with Dynabeads™ RNA Binding Buffer (Part B) (see “Procedure overview” on page 1).

Note: This guide can also be used with Cat. No. 49010D, 49011D, 49020D, 49021D, 49040D, and 49041D, which are available through OEM/commercial supply.

Procedure overview

Part A—A biotinylated template is produced by PCR amplification of the target sequence in a plasmid or synthetic DNA construct. This process uses a biotinylated forward primer positioned at least 30–100 base pairs upstream of the T7 promoter and a non-biotinylated reverse primer. Greater distance between the T7 promoter and the forward primer is beneficial when designing qPCR assays to evaluate template leaching. Alternatively, a linearized plasmid can be biotinylated through a fill-in reaction using biotin-dUTP in the 5' overhang sequence, contingent on correct plasmid design. The biotinylated template is then directly immobilized onto Dynabeads™ Streptavidin for In Vitro Transcription beads without requiring previous purification. Following immobilization, the bead-bound template is used directly in IVT, then removed from the synthesized RNA by magnetic separation (see Figure 1). The bead-bound template can be stored for reuse at -20°C or -80°C for up to one month in TE or PBST.

Part B—The crude RNA produced by IVT is mixed with Dynabeads™ Carboxylic Acid for RNA Purification beads, then the Dynabeads™ RNA Binding Buffer is added to the mixture. With the RNA bound to the surface of the beads, the remaining components of the reaction mixture are removed by applying a magnet, then discarding the supernatant (see Figure 1).

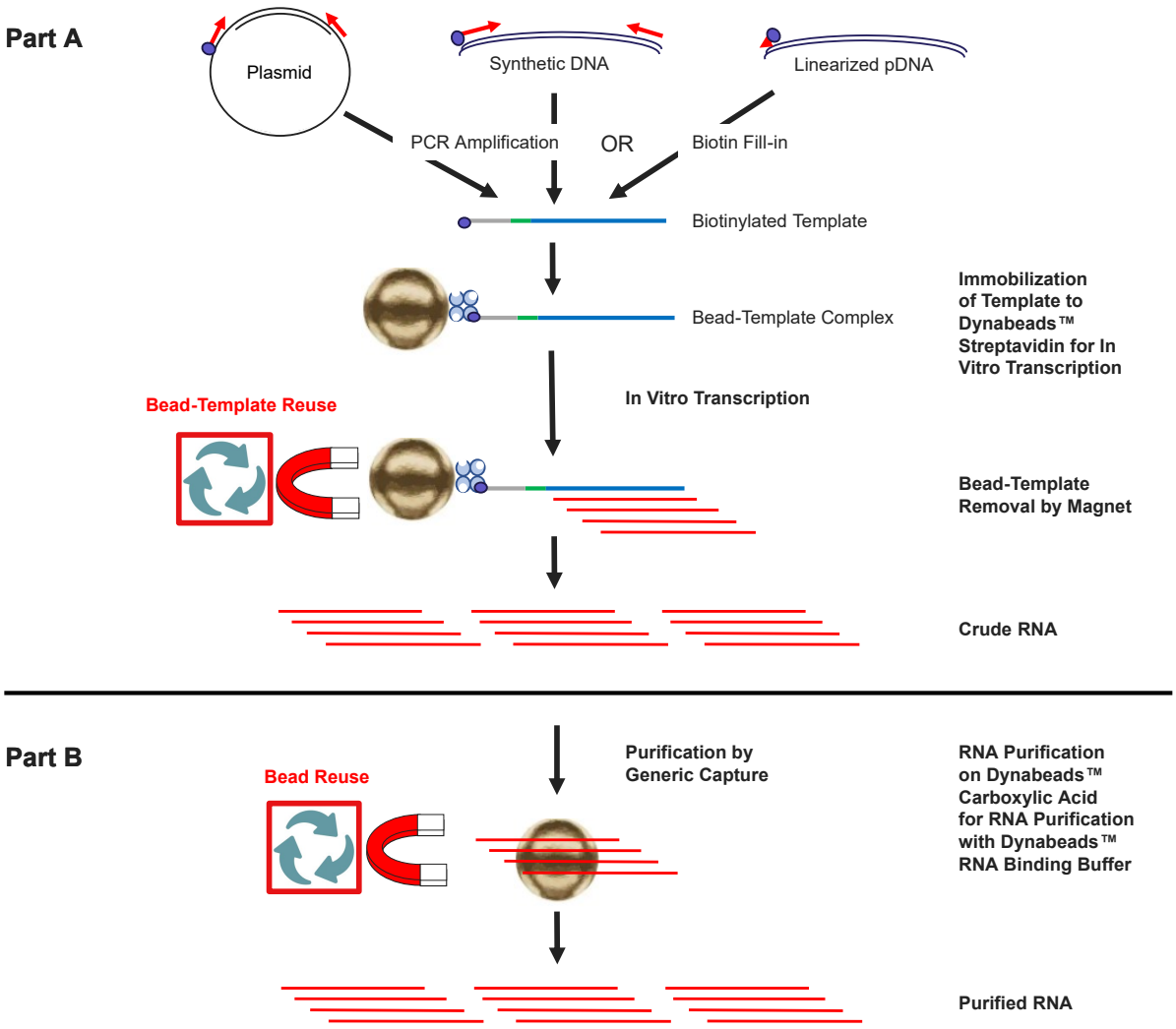

Figure 1 Workflow for RNA synthesis with Dynabeads™ Streptavidin for In Vitro Transcription beads and RNA purification using Dynabeads™ Carboxylic Acid for RNA Purification beads

- **Part A—**IVT of a biotinylated DNA template that is immobilized onto Dynabeads™ Streptavidin for In Vitro Transcription beads
- **Part B—**Purification of the crude RNA produced by IVT using Dynabeads™ Carboxylic Acid for RNA Purification beads in combination with Dynabeads™ RNA Binding Buffer

Note: The beads from Part A and Part B can be reused at least six times.

Contents and storage

Item	Cat. No.	Amount	Storage
Part A			
Dynabeads™ Streptavidin for In Vitro Transcription[1]	65005D	2 mL	2–8°C
	65005D	10 mL	

Item	Cat. No.	Amount	Storage
Part B			
Dynabeads™ Carboxylic Acid for RNA Purification[2]	65020D	2 mL	2–8°C
	65021D	10 mL	
Dynabeads™ RNA Binding Buffer	65040D	20 mL	2–8°C Protect from light.
	65042D	50 mL	

[1] Supplied at a 10 mg/mL concentration in PBS buffer with 0.01% Tween 20 and 20% ethanol added as preservatives.
[2] Supplied at a 10 mg/mL concentration in purified water.

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
Parts A and B	
DynaMag™-2 Magnet	thermofisher.com/magnets
Thermal mixer [1]	13687717
UltraPure™ DNase/RNase-Free Distilled Water	10977035
RNase-free tubes (for example, RNase-free Microfuge Tubes, 1.5 mL)	AM12400
Qubit™ RNA BR Assay Kit	Q10210
Part A	
Tris (1 M), pH 8.0, RNase-free	AM9855G
2X Streptavidin Binding and Washing Buffer (10 M Tris HCl, pH 7.5, 1 mM EDTA, 2 M NaCl, 0.1% Tween 20)	MLS
Tween™-20 Surfact-Amps™ Detergent Solution (10%)	28321
IVT kit of choice (for example, Invitrogen™ MEGAscript™ T7 Transcription Kit)	AMB13345
TheraPure™ and TheraPure™ GMP mRNA product lines	thermofisher.com/therapure
Platinum™ SuperFi™ II PCR Master Mix	12368010
Anza™ 33 LguI	IVGN0334
Biotin-11-dUTP Solution (1 mM)	R0081
Klenow Fragment, exo– (5 U/µL)	EP0421
Part B	
70% ethanol (wash solution)[2]	MLS
TE, pH 7.0, RNase-free	AM9861
Tris (1 M), pH 7.0, RNase-free	AM9850G

[1] Thermal mixer with tilting and/or rotation of tubes to keep beads in suspension during template immobilization and the IVT reaction.
[2] Prepare using UltraPure™ DNase/RNase-Free Distilled Water.

Guidelines for producing the biotinylated template

- To produce a biotinylated template by PCR, use a high-fidelity system, such as Platinum™ SuperFi™ II PCR Master Mix (Cat. No. 12368010).
  - Introduce the poly-A tail either during PCR with the reverse primer (with poly T-tail) or as part of the sequence. Position the T7-promoter upstream of the 5' UTR (untranslated region) and the ORF (Open Reading Frame). Alternatively, a defined poly-A-tail can be incorporated at the 3' end.

– For successful solid-phase IVT, ensure that the forward primer is biotinylated, HPLC-purified, and maintains a distance of at least 30–100 bp from the T7-promoter.

Note: If your are designing an upstream qPCR amplicon to quantify potential template leaching in the IVT reaction, it can be beneficial to position the forward primer further upstream from the T7 promoter. Such leaching, however, is typically minimal and requires qPCR for detection.

- To produce the biotinylated template from a plasmid, linearize the plasmid using LguI (or BspQI/SapI), immediately after the poly A-tail, then biotinylate using a fill-in reaction of biotin-dUTP in the 5' overhang sequence (see "Prepare a biotinylated template from a plasmid" on page 5).
  We recommend using Anza™ 33 LguI (Cat. No. IVGN0334) for plasmid linearization, and Biotin-11-dUTP Solution (Cat. No. R0081) and Klenow Fragment, exo– (5 U/µL) (Cat. No. EP0421) for the biotin fill-in reaction.

Guidelines for preparing the biotinylated template mix

- For a 100 µL IVT reaction, use the following amount of biotinylated template as a starting point:
  – **For a biotinylated PCR product—**Use 1–2 µg of a 2.5 kb biotinylated PCR product that is immobilized to 1 mg of Dynabeads™ Streptavidin for In Vitro Transcription beads. This results in a final template concentration of 6.1–12.1 nM in the IVT reaction.
  – **For a biotinylated plasmid template—**Use 1.6–3.2 µg of 4 kb biotinylated plasmid template that is immobilized to 1 mg of Dynabeads™ Streptavidin for In Vitro Transcription beads. This results in a final template concentration of 6.1–12.1 nM in the IVT reaction.

  Note: Further optimization may be required for different template sizes.
- For optimal RNA yield, ensure that the density of the biotinylated template on the bead surface is between 0.5–1 pmol/mg beads. Do not use more than 4 µg of 2.5 kb template/mg beads (this corresponds to a template density of 2 pmol/mg beads). Excessive template density can result in a decrease in RNA yield.

Guidelines for IVT

- IVT reaction volumes can be scaled up or down in a linear fashion by adjusting all components of the reaction proportionally.
- Dynabeads™-based solid-phase IVT is compatible with reagents, such as Invitrogen™ MEGAscript™ T7 Transcription Kit, Invitrogen™ mMESSAGE mMACHINE™ T7 Transcription Kit, Invitrogen™ mMESSAGE mMACHINE™ T7 ULTRA Transcription Kit, and the TheraPure™ and TheraPure™ GMP product lines based on the needs of the user and the strategies for capping and polyadenylation.

Before you begin

Prepare buffers

Prepare buffers as described in the following table, using UltraPure™ DNase/RNase-Free Distilled Water in sterile, RNase-free tubes or bottles.

IMPORTANT! Autoclave or sterile-filter 2X Streptavidin Binding and Washing Buffer before use.

Buffer	Components
2X Streptavidin Binding and Washing Buffer (stock solution)	• 10 mM Tris-HCl, pH 7.5 • 1 mM EDTA • 2 M NaCl • 0.1% Tween 20
1X Streptavidin Binding and Washing Buffer	• 50 mL 2X Streptavidin Binding and Washing Buffer • 50 mL UltraPure™ DNase/RNase-Free Distilled Water
10 mM Tris, pH 8, 0.05% Tween 20, Washing Buffer **Note:** Prepare fresh on each day of use (recommended).	• 1 mL Tris (1 M), pH 8, RNase-free • 0.5 mL Tween 20 (from 10% solution) • 98.5 mL UltraPure™ DNase/RNase-Free Distilled Water

Prepare a biotinylated template from a plasmid

The following procedure describes the plasmid linearization and biotin fill-in reactions to produce a biotinylated plasmid template. If you are using a biotinylated PCR product, proceed to prepare the biotinylated template mix (next section).

1. Add the following reagents to an RNase-free tube.

   Table 1 Plasmid linearization reaction (final volume = 100 µL)

Reagent	Volume per reaction
Plasmid DNA (50 µg)	X µL
Anza™ 33 LguI	2 µL
Anza™ 10X Buffer [1]	10 µL
UltraPure™ DNase/RNase-Free Distilled Water	to 100 µL

[1] Component of Anza™ 33 LguI (Cat. No. IVGN0334)

2. Pipet up and down several times to mix the reagents, then incubate for 3 hours at 37°C.

3. Centrifuge the tube for 5 seconds to collect the contents. Ensure that the lid of the tube is free of condensate following centrifugation.

   The linearized plasmid is in the tube.

4. Add the following reagents to a new RNase-free tube.

   Table 2 Biotin fill-in reaction of biotin-dUTP in the 5-prime overhang sequence

Reagent	Volume per reaction
Linearized plasmid	100 µL
Biotin-11-dUTP Solution	5 µL
Klenow Fragment, exo– (5 U/µL)	2 µL

5. Pipet up and down several times to mix the reagents, then incubate for 1 hour at 37°C.

6. Incubate the biotin fill-in reaction for 10 minutes at 75°C, then centrifuge the tube for 5 seconds. Place on ice.

7. To determine the final concentration of linearized and biotinylated plasmid, use the amount of template input in the reaction, giving a final concentration of 0.467 µg/µL.

   Note: Quantification of the linearized and biotin fill-in plasmid concentration can be challenging. The final concentration can be calculated from the amount of plasmid input in the reaction.

Prepare the biotinylated template mix

Add the following reagents, in the order indicated, to an RNase-free tube. Ensure a final 1X concentration of Streptavidin Binding and Washing Buffer.

Table 3 Preparation of biotinylated template mix for an IVT reaction volume of 100 µL/mg of Dynabeads™ Streptavidin for In Vitro Transcription beads

Reagent	Amount per 100-µL IVT reaction
Biotinylated template	1–2 µg of PCR product (2.5 kb) or 1.6–3.2 µg of plasmid (4.0 kb)
UltraPure™ DNase/RNase-Free Distilled Water	add to total 50 µL
2X Streptavidin Binding and Washing Buffer	50 µL
Final volume of biotinylated template mix	**100 µL**

Part A: RNA synthesis with Dynabeads™ Streptavidin for In Vitro Transcription beads

Ensure the following buffers are prepared before starting this procedure:

- 2X Streptavidin Binding and Washing Buffer (10 mM Tris-HCL, pH 7.5, 1 mM EDTA, 2 M NaCl, 0.1% Tween 20)
- 1X Streptavidin Binding and Washing Buffer (5 mM Tris-HCL, pH 7.5, 0.5 mM EDTA, 1 M NaCl, 0.05% Tween 20)
- 10 mM Tris, pH 8, 0.05% Tween 20

1 Prepare Dynabeads™ Streptavidin for In Vitro Transcription beads

1. Vortex the Dynabeads™ Streptavidin for In Vitro Transcription beads thoroughly to ensure that the beads are fully resuspended.

   Note: Ensure that the beads remain fully dispersed in the solution during pipetting.

2. Place the beads on a roller for at least 20 minutes at room temperature.
3. Transfer 100 µL (1 mg) of the resuspended beads to an RNase-free tube.
4. Place the tube on a magnet for 15–30 seconds, then discard the supernatant.
5. Wash the beads—Add 100 µL of 1X Streptavidin Binding and Washing Buffer, then pipet up and down several times or briefly vortex to resuspend the beads.

2 Immobilize the biotinylated template to the beads

1. Place the tube containing the prepared Dynabeads™ Streptavidin for In Vitro Transcription beads on the magnet for 15 seconds, then discard the supernatant.
2. Add 100 µL of biotinylated template mix to the bead pellet, then pipet up and down several times to thoroughly resuspend the beads.
3. Incubate on a thermal mixer set at 1,500 RPM for 30 minutes at room temperature to immobilize the biotinylated template to the beads.
4. Place the tube containing the DNA-bead complex on the magnet for 15–30 seconds, then discard the supernatant.
5. Wash the DNA-bead complex—Add 100 µL of 10 mM Tris, pH 8, 0.05% Tween 20, then pipet up and down several times or briefly vortex to resuspend the beads. Place the tube on the magnet for 15 seconds, then discard the supernatant.
6. Repeat step 2.5 three times for a total of four washes. Do not remove the supernatant after the last wash.

3 Prepare the IVT reaction mix

IMPORTANT! Keep all the reagents of the MEGAscript™ T7 Transcription Kit on ice during use, except the 10X IVT Reaction Buffer.

Combine the following reagents from the MEGAscript™ T7 Transcription Kit in the order indicated.

Table 4 Preparation of the IVT reaction mix for manual and automated workflows

Reagent	Volume per reaction	
	Manual workflow: 20 µL reaction (1X)	Automated workflow: 100 µL reaction (5X)[1]
Nuclease-free water[2]	8 µL	40 µL
ATP	2 µL	10 µL
CTP	2 µL	10 µL
GTP	2 µL	10 µL
UTP	2 µL	10 µL
10X Reaction Buffer	2 µL	10 µL
Enzyme Mix	2 µL	10 µL

3 *(continued)*

Reagent	Volume per reaction	
	Manual workflow: 20 µL reaction (1X)	Automated workflow: 100 µL reaction (5X)[1]
Template	Bead pellet[3]	Bead pellet[3]
Total volume of IVT reaction mix per reaction	**20 µL**	**100 µL**

[1] The reaction is directly scalable by increasing or decreasing all components proportionally.
[2] Included in the kit.
[3] The volume of the bead pellet does not need to be subtracted.

4 Perform the IVT reaction

The following procedure uses the DNA-bead complex that was prepared in a previous section (see "Immobilize the biotinylated template to the beads" on page 6).

1. Place the tube containing the DNA-bead complex on the magnet, then discard the supernatant.
2. Immediately add 100 µL of the IVT reaction mix, then pipet up and down several times to thoroughly resuspend the DNA-bead complex.

   IMPORTANT! Do not allow the DNA-bead complex to dry.

3. Incubate on a thermal mixer set at 1,500 RPM for 1–3 hours at 37°C .

   Note: The reaction time in this step should be optimized for each template and desirable RNA yield.

4. Place the tube on the magnet for 15–30 seconds, then transfer the supernatant containing the RNA to a new, RNase-free tube.

   Note:
   - This step removes the template from the synthesized RNA, because the template remains immobilized on the bead surface.
   - The DNA-bead complex can be reused at least six times in consecutive IVT reactions.

5. Place the tube containing the RNA on ice before analysis or freeze at -80°C.
6. Determine the RNA concentration. We recommend using the Qubit™ RNA BR Assay Kit to determine RNA yield.

   Note: The RNA yield per milligram of beads depends on several parameters, such as the template density that is immobilized on the bead surface and the length of the IVT reaction. A 100 µL 2-hour reaction typically yields 4 µg/µL RNA of 2.5 kb biotinylated template, giving up to 400 µg per reaction. The RNA yield can vary between different biotinylated templates.

Store the DNA-bead complex for reuse at -20°C or -80°C for up to one month in TE or PBST. Before reuse, wash the DNA-bead complex in 10 mM Tris, pH 8, 0.05% Tween 20. If the IVT yield is low, add Tween 20 to the IVT reaction mix at a final concentration of 0.05%.

Recommended starting volumes for RNA purification

We recommend using equivalent volumes of Dynabeads™ RNA Binding Buffer and input RNA solution. To scale this procedure, keep the ratio of RNA and bead volumes constant; the relative volumes of wash solution can be adjusted.

Use the following experimental set-up as a starting point. Further optimization may be required.

Table 5 Recommended starting volumes for RNA purification

Reagent	Volume
Crude IVT mix (input RNA solution)	100 µL[1]
Dynabeads™ Carboxylic Acid for RNA Purification beads	30 µL (300 µg)
Dynabeads™ RNA Binding Buffer	100 µL
Elution output	100 µL

[1] For 2,500 nt-long RNA, we have tested concentrations up to 5 µg/µL with over 90% recovery.

Part B: RNA purification using Dynabeads™ Carboxylic Acid for RNA Purification beads with Dynabeads™ RNA Binding Buffer

The following section describes a general procedure for capture purification of RNA following the IVT reaction. The purification removes NTPs, proteins, and other remaining components of the IVT reaction mix.

1 Prepare the RNA samples and Dynabeads™ Carboxylic Acid for RNA Purification beads

1. If needed, dilute the RNA sample to ≤5 µg/µL with 10 mM Tris-HCl, pH 7.0. Place on ice.
2. Vortex the Dynabeads™ Carboxylic Acid for RNA Purification beads for 10 seconds to ensure that the beads are fully resuspended.

   Note: Ensure that the beads remain fully dispersed in the solution during pipetting.
3. Place the beads on a roller for at least 20 minutes at room temperature.
4. For each sample to be purified, transfer 30 µL (300 µg) of the resuspended beads to an RNase-free tube.
5. Place the tube containing the beads on a magnet until the supernatant is clear (about 30 seconds), then discard the supernatant.
6. Wash the beads—Add 100 µL of UltraPure™ DNase/RNase-Free Distilled Water.
7. Place the tube on the magnet, then discard the supernatant.

2 Bind the RNA to the beads

1. For each sample to be purified, add 100 µL of the prepared RNA sample to the bead pellet (300 µg), then pipet up and down several times to thoroughly resuspend the beads.
2. Add 100 µL of Dynabeads™ RNA Binding Buffer, then gently pipet up and down until the solution is homogeneous.
3. Incubate on a thermal mixer set at 1,000 RPM for 10 minutes at room temperature .
4. Place the tube on a magnet until the supernatant is clear (about 1-2 minutes), then discard the supernatant.

3 Wash, then dry the RNA-bead complex

1. Add 500 µL of wash solution (70% ethanol) to the RNA-bead complex, then briefly vortex to resuspend the beads.

   Note: Some aggregation of the beads during the washing steps is expected.
2. Place the tube on a magnet until the supernatant is clear (about 30–60 seconds), then discard the supernatant.
3. Repeat step 3.1 and step 3.2 two times for a total of three washes.

3 *(continued)*

4. After the last wash, keep the tube on the magnet, then discard any remaining wash solution in the tube or the lid.
5. Keep the tube on the magnet with the lid open, then allow the bead pellet to dry for 10 minutes at room temperature.

   Note: Ensure that the tube lid is open during the drying step and that no wash solution remains after 10 minutes.

4 **Elute the RNA**

1. Remove the tube from the magnet, then add 100 µL of Elution Buffer (TE buffer, pH 7, or another low-salt buffer of choice).
2. Pipet up and down to resuspend the beads.
3. Incubate on a thermal mixer set at 1,000 RPM for 5 minutes at room temperature .
4. Place the tube on the magnet, then transfer the cleared supernatant containing the purified RNA to a new RNase-free tube.
5. Place the tube on ice, then proceed with analyses.
6. Determine the RNA concentration. We recommend using the Qubit™ RNA BR Assay Kit or NanoDrop™ One Microvolume UV-Vis Spectrophotometer.

Troubleshooting

Observation	Possible cause	Recommended action
Low yield of IVT RNA	A low amount of template was immobilized.	Quantify the amount of template in the unbound fraction. The typical template immobilization rate is >85%.
		The efficiency of biotinylation of the template was too low. For biotinylated PCR products, use HPLC-purified biotinylated primer in the PCR.
	The IVT reaction time was insufficient.	Optimize the IVT reaction time for your template and the IVT kit used.
	Reagents were not added to the IVT reaction mix in the correct order.	Add the reagents in the same order as described. See “Prepare the IVT reaction mix” on page 6.
	The temperature of the thermal mixer heating block during the IVT reaction was not at an optimal level. An IVT temperature that is too high or too low can decrease the IVT yield.	To ensure the optimal temperature for the IVT reaction, use a probe thermometer (such as the Fluke 54 II) to determine the well temperature at intervals of 5, 10, 15, 20, and 25 minutes. Adjust the heating block set temperature accordingly. Ensure that the volume of the well is the same as the volume of the IVT reaction.
	Different IVT kits can give variable RNA yield, depending on the template.	Test different IVT kits for the desired RNA yield. A control template in the IVT kit can be used as a standard.
	RNase contaminated the reagents.	Use sterile tubes. Prepare RNase-free buffers, using RNase-free reagents.
	The DNA-bead complex was not properly prepared before reuse.	Wash the DNA-bead complex with 10 mM Tris, pH 8, 0.05% Tween 20 before reuse. Add Tween 20 in the IVT reaction mix to a final concentration of 0.05%.
Low RNA recovery	RNase contaminated the reagents.	Use sterile and RNase-free tubes, pipette tips, reagents, and buffers.
	RNA precipitated in solution.	Do not premix the crude RNA and Dynabeads™ RNA Binding Buffer before adding the Dynabeads™ Carboxylic Acid for RNA Purification beads to prevent RNA precipitation, which can decrease RNA recovery. Follow the procedure as described—premix the crude RNA with the beads before adding the Dynabeads™ RNA Binding Buffer.
	The size of the RNA was not optimal.	Purification recovery can vary depending on the size of the RNA. To optimize the protocol for your specific RNA size, adjust the RNA input or the amount of Dynabeads™ Carboxylic Acid for RNA Purification beads.

Observation	Possible cause	Recommended action
Too high RNA recovery	The Qubit™ RNA BR Assay Kit measurement was inaccurate.	The recovery rate can exceed 100% when the measurement of input RNA is lower (due to an unknown inhibition factor) compared to the amount of purified RNA.

Limited product warranty

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Revision history: Pub. No. MAN0029518 B

Revision	Date	Description
B	4 September 2024	• New items were added to the required materials list, including TheraPure™ and TheraPure™ GMP mRNA product lines. • New instructions were added for preparing buffers, biotinylated template from a plasmid, and biotinylated template mix. • A troubleshooting section was added. • Minor updates were made throughout for consistency of style and terminology. • Version numbering was changed in conformance with internal document control procedures.
A.0	10 July 2023	New document for Dynabeads™-Based Solid-Phase In Vitro Transcription and RNA Purification.