invitrogen

MEGAclear™ dsRNA Removal Kit

USER GUIDE

Catalog Numbers A56871, A56872

Publication Number MAN1002230

Revision B

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

Thermo Fisher Scientific Baltics UAB | V.A. Graiciuno 8, LT-02241 | Vilnius, Lithuania
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN1002230 B (English)

Revision	Date	Description
B	11 August 2026	Rev. B: Minor changes to the "Contents and Storage" section.
A	30 May 2026	New document for MEGAclear™ dsRNA Removal Kit.

The information in this guide is subject to change without notice.

Contents

1 Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

A significant challenge in producing high-quality *in vitro* transcribed (IVT) mRNA is the by-product generation of double-stranded RNA (dsRNA, Figure 1). dsRNA can be in two forms: 1) smaller than full-length, which is thought to be formed by aborted transcripts annealing to mRNA and extending to form truncated RNA products or 2) larger than full length where the RNA polymerase extends a full-length RNA product that has looped back and annealed to itself.

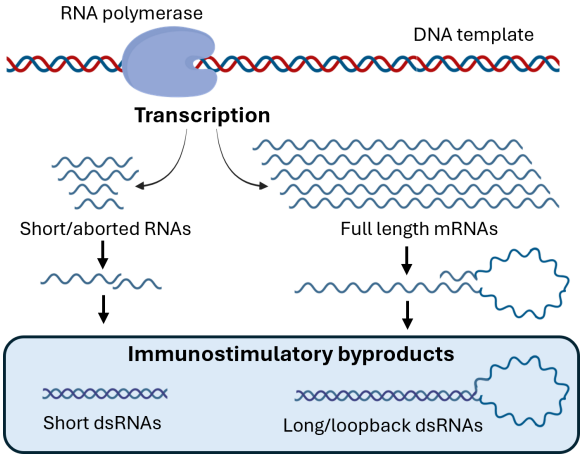

Figure 1 Mechanism of dsRNA formation in IVT reactions. The RNA polymerase can use RNA as a template, resulting in dsRNA formation.

This contaminant stimulates innate immune receptors, including Toll-like receptors and cytosolic sensors, leading to reduced translation, inflammatory responses, and cytotoxicity.

The MEGAclear™ dsRNA Removal Kit contains all the reagents and buffers necessary to remove dsRNA from *in vitro* transcribed mRNA synthesis reactions. One reaction will remove dsRNA from ~100 µg IVT mRNA. Reactions can be scaled up linearly to remove dsRNA from up to 10 mg IVT mRNA. This kit can reduce dsRNA to below the quantifiable range of commonly used dsRNA ELISA assays with ≥75% mRNA recovery.

MEGAclear™ dsRNA Removal Kits employ a specialized dsRNA-affinity protein that selectively captures and removes dsRNA contaminants while preserving the integrity of single-stranded mRNA. This step is performed with the completed and unpurified IVT reaction, supporting a rapid and effective polishing step that removes dsRNA contaminants prior to mRNA purification.

IMPORTANT!

- This kit removes only dsRNA from IVT mRNA. A final purification step is required after the dsRNA removal step to remove the IVT proteins and excess nucleotides. The following kits or reagents can be used for the mRNA purification:
  - MEGAclear™ Transcription Clean-Up Kit (Cat. No. AM1908).
  - Dynabeads™ RNA Purification kit (Cat. No. 65030D).
  - LiCl precipitation which are included in our IVT kits or can be purchased separately (Cat. No. AM9480).
- This kit is intended to be used with our mMESSAGE mMACHINE™ T7 mRNA kit with CleanCap™ Reagent AG (Cat. No. A57620-25) and N1-methylpseudouridine (m1Ψ) which is the most commonly used modification. It may be used with other IVT kits or uridine modifications; however, the protocol might require adjustment and experimental testing to achieve the desired dsRNA removal efficiency and/or mRNA recovery. **See "Guidance for alternate use of the kit" on page 14 for guidance.**

Contents and storage

The kit has components that require different storage temperatures. The dsRNA Binding Protein should be stored at –20°C in a non-frost-free freezer. The 10X Binding Buffer and dsRNA Capture Beads should be stored at 2–8°C.

Table 1 Kit components, volumes, and storage conditions.

Component	Amount		Storage [1]
	25 reactions kit	1000 reactions kit	
MEGAclear™ dsRNA Binding Protein	0.125 mL	5 mL	–25°C to –15°C
MEGAclear™ 10X Binding Buffer	0.75 mL	30 mL	2–8°C
MEGAclear™ dsRNA Capture Beads	2.5 mL	100 mL	

[1] When using the kit, keep the protein on ice and bring the buffer and beads to room temperature.

Required materials not supplied

- Nuclease-free water (example: UltraPure Water Cat. No. 10977015).
- Magnetic bead stand. This kit works with our DynaMag™ Magnets:
  - DynaMag™–2 Magnet (Cat. No. 12321D) for a standard reaction.
  - DynaMag™–15 (Cat. No. 12301D) or DynaMag™–50 (Cat. No. 12302D) Magnet for larger scale reactions.
  - DynaMag™–96 Side (Cat. No. 12331D) or DynaMag™–96 Side Skirted (Cat. No. 12027) Magnet for high-throughput applications.
  - This kit has not been tested with other magnets. The separation times of other magnet sources may vary which can impact mRNA purity. The protocol might require adjustment and experimental testing to achieve the desired dsRNA removal efficiency and/or mRNA recovery if using other magnet sources.
- IVT mRNA:
  - This kit has been designed to be used with our mMESSAGE mMACHINE™ T7 mRNA kit with CleanCap™ Reagent AG (Cat. No. A57620-25) and MEGAscript™ T7 Transcription Kit Plus (Cat. No. A57622-25).
  - It may be used with other IVT kits; however, the protocol might require adjustment and experimental testing to achieve the desired dsRNA removal efficiency and/or mRNA recovery. **See "Guidance for alternate use of the kit"** on page 14 **for guidance**.
- mRNA purification kit. For final purification of the synthesized mRNA, we recommend:
  - MEGAclear™ Transcription Clean-Up Kit (Cat. No. AM1908) for column-based quick cleanup and small purification scales (eg: 100 µg).
  - Dynabeads™ RNA Purification Kit (Cat. No. 65030D) is designed for magnetic bead–based cleanup and is compatible with automated workflows or high-throughput applications.
  - LiCl Precipitation Solution (supplied with our IVT kits or Cat. No. AM9480) provides a cost-effective method for mRNA concentration and is compatible with a wide range of scales.

2 Methods

MEGAclear™ dsRNA removal kit procedure (for 100 µg IVT reaction)

Table 2 Overview of the mRNA synthesis and purification workflow with required kits or materials.

Step	Process	Estimated Time[1]	Required Kits / Materials
1	Perform *in vitro* transcription to produce mRNA, followed by DNase incubation to remove the DNA template	2 hours and 30 minutes	mMessage mMACHINE™ T7 mRNA Kit with CleanCap™ Reagent AG (Cat. No. A57620-25). or MEGAscript™ T7 Transcription Kit Plus (Cat. No. A57622-25) **Note:** Other IVT kits may be used; however, protocol adjustments and experimental optimization may be required to achieve the desired dsRNA removal efficiency and/or mRNA recovery. **See "Guidance for alternate use of the kit"** on page 14 **for guidance**.
2	Add dsRNA Binding Protein and dsRNA Capture Beads	18 minutes	MEGAclear™ dsRNA Removal Kit, (Cat. No. A56871, A56872).
3	Remove dsRNA-bound beads by magnet	2 minutes	• DynaMag™–2 Magnet (Cat. No. 12321D) for a standard reaction. • DynaMag™–15 (Cat. No. 12301D) or DynaMag™–50 (Cat. No. 12302D) Magnet for larger scale reactions. • DynaMag™–96 Side (Cat. No. 12331D) or DynaMag™–96 Side Skirted (Cat. No. 12027) Magnet for high-throughput applications.

Table 2 Overview of the mRNA synthesis and purification workflow with required kits or materials. *(continued)*

Step	Process	Estimated Time[1]	Required Kits / Materials
4	Perform mRNA purification	Varies depending on purification strategy	One of the following mRNA purification kits can be used: • MEGAclear™ Transcription Clean-Up Kit (Cat. No. AM1908). • Dynabeads™ RNA Purification Kit (Cat. No. 65030D) and DynaMag™ (See above). • LiCl Precipitation Solution (supplied with our IVT kits or Cat. No. AM9480). • *Not required but recommended:* The RNA Storage Solution (Cat. No. AM7000 or AM7001) **Note:** Other purification kits/methods may be used; however, the protocol might require adjustment and experimental testing to achieve the desired dsRNA removal efficiency and/or mRNA recovery.

[1] Estimated time is based on a single 100 µg reaction and includes bead preparation during the DNase step. Additional reactions or larger scales will increase total processing time.

Upstream IVT mRNA reaction

1. Refer to the user guide for the mMESSAGE mMACHINE™ T7 Transcription Kit with CleanCap™ Reagent AG (Pub. No. MAN0030027) for mRNA synthesis at the 100 µg / 20 µL reaction scale.
2. After adding DNase to the completed IVT reaction, bring the 10X Binding Buffer and dsRNA Capture Beads to room temperature. Proceed to the dsRNA Removal Procedure after the DNase step.

Note: For faster processing, prepare the beads as described in Step 3 of the next section during the DNase incubation. Leave the beads in the second wash until ready to add the sample.

dsRNA removal procedure

1. In a nuclease-free tube, thoroughly mix the components in the order listed in the following table:

Amount	Component
21 µL	IVT reaction + DNase
64 µL	Nuclease-free Water
10 µL	10x Binding Buffer
5 µL	dsRNA Binding Protein

2. Incubate at room temperature while preparing the dsRNA Capture Beads (Step 3).

   Note: If the beads are prepared in advance, incubate the sample at room temperature for at least 5 minutes, no longer than 30 minutes.

3. Meanwhile, prepare the dsRNA Capture Beads as follows:
   a. Prepare 1X Binding Buffer by mixing 22 µL 10X Binding Buffer with 198 µL nuclease-free water.

   b. Vortex the magnetic beads thoroughly to fully resuspend the beads. Ensure there are no clumps at the bottom, and that the beads remain fully dispersed during pipetting.

   c. After the beads are thoroughly resuspended, immediately transfer 100 µL of bead slurry to a clean nuclease-free microtube.

   d. Place the microtube on the magnetic stand and wait 2 minutes until the beads cluster against the tube walls and the solution clears.

   e. Remove and discard the storage buffer, taking care not to disturb the bead pellet.

   f. First wash - Remove the microtube from the magnet and wash the beads with 100 µL 1X Binding Buffer. Pipette up and down gently several times to thoroughly mix with the beads, taking care not to introduce bubbles.

   g. Place the microtube on the magnetic stand and wait 2 minutes until the solution clears.

   h. Remove and discard the supernatant, taking care not to disturb the bead pellet.

   i. Second wash - Remove the microtube from the magnet and wash the beads with 100 µL 1X Binding Buffer. Pipette up and down gently several times to thoroughly mix with the beads, taking care not to introduce bubbles.

   j. Place the microtube on the magnetic stand and wait 2 minutes until the solution clears.

   k. When ready to perform the next Step (4), remove and discard the supernatant, taking care not to disturb the bead pellet.

4. After the mixture from Step 1 has incubated for at least 5 minutes and the final bead wash has been removed, take the microtube containing the beads off the magnet. Immediately transfer the entire mixture from Step 1 to the beads. Mix thoroughly by pipetting up and down several times, taking care not to introduce bubbles. Ensure no bead clumps remain in the mixture or on the tube walls

5. Incubate at room temperature for 5 minutes, gently flicking the microtube every 2 minutes to prevent the beads from settling on the bottom.

6. Incubate at 37°C for 5 minutes. This step helps reduce nonspecific interactions between the mRNA and the magnetic beads.

7. Place the microtube on the magnetic stand and wait 2 minutes until the beads cluster against the tube walls and the solution clears.

8. Transfer the **supernatant** (~100 µL) to a clean nuclease-free tube. This contains the mRNA and needs to be purified immediately as described in the next section **RECOVERY OF THE RNA**. The dsRNA remains bound to the bead pellet and can be discarded.

Recovery of the RNA

This section provides guidance for purifying the mRNA following the dsRNA removal step using one of the following methods:

1. MEGAclear™ Transcription Clean-Up Kit (Cat. No. AM1908).
2. Dynabeads™ RNA Purification Kit (Cat. No. 65030D).
3. LiCl Precipitation Solution (Cat. No. AM9480).

MEGAclear™ Transcription Clean-Up Kit

This kit is designed for purifying mRNA from high-yield *in vitro* transcription reactions. The procedure efficiently removes nucleotides, short oligonucleotides (<100 nucleotides), proteins, and salts from the mRNA. The purified mRNA is suitable for applications requiring high purity.

The binding capacity of a MEGAclear™ column is 500 µg mRNA; therefore, multiple columns are required for input amounts >500 µg.

Following dsRNA-bead removal, proceed according to the MEGAclear™ Transcription Clean-Up Kit User Guide (Cat No. AM1908). The supernatant (~100 µL) from the dsRNA cleanup step corresponds to the required input volume. Add Binding Solution Concentrate (350 µL) to the supernatant and continue as described in the user guide.

The mRNA may be eluted using THE RNA Storage Solution (Cat. No. AM7000 or AM7001), which provides greater RNA stability than standard 0.1 mM EDTA or TE buffer.

Dynabeads™ RNA Purification Kit

The Dynabeads™ RNA Purification Kit provides a simple, flexible, and automation-ready solution for purifying RNA from IVT reactions.

Following dsRNA-bead removal, proceed according to the Dynabeads™ RNA Purification Kit User Guide (Part B: RNA purification using Dynabeads™ Carboxylic Acid beads and RNA Binding Buffer .

The supernatant (~100 µL) from the dsRNA cleanup step corresponds to the required input volume. Add the supernatant to 30 µL of washed Dynabeads™ Carboxylic Acid bead slurry and proceed as described in the user guide.

The mRNA may be eluted using the RNA Storage Solution (Cat. No. AM7000 or AM7001), which provides greater RNA stability than standard 0.1 mM EDTA or TE buffer.

LiCl Precipitation Solution

Lithium Chloride (LiCl) precipitation may be used to purify RNA by removing unincorporated nucleotides and most proteins. However, LiCl precipitation may not efficiently recover RNAs smaller than 300 nucleotides. For efficient precipitation, the RNA concentration should be at least 0.1 µg/µL

A LiCl Precipitation Solution is included with the mMESSAGE mMACHINE™ T7 mRNA Kit with CleanCap™ Reagent AG and is also available separately as a 100 mL solution (Cat. No. AM9480). To precipitate mRNA following the dsRNA-bead removal step, perform the following:

1. Add 100 µL LiCl Precipitation Solution to the supernatant (~100 µL) from the dsRNA cleanup step.
2. Mix thoroughly and incubate at –20°C for 30–60 minutes.
3. Centrifuge at 4°C for 15 minutes at maximum speed (for example, 16,000–17,000 × g) to pellet the RNA.
4. Carefully remove the supernatant. Wash the pellet once with ~1 mL cold 70% ethanol, then centrifuge again at 4°C for 5 minutes at maximum speed to maximize removal of unincorporated nucleotides.
5. Carefully remove the ethanol and allow the pellet to air-dry at room temperature for 5 minutes.
6. Resuspend the RNA in 50–100 µL nuclease-free water, RNA Storage Solution (Cat. No. AM7001), or TE buffer.

Quantitation of purified mRNA products

Quantitation by UV light absorbance

Reading the absorbance at 260 nm (A260) of RNA sample is the simplest way to determine yield, but any unincorporated nucleotides and/or template DNA in the mixture will contribute to the reading. Therefore, we recommend purification of the RNA product before measuring A260. The RNA yield can be calculated as follows:

- A260 × 40 = µg/mL RNA

The Thermo Scientific™ NanoDrop Microvolume Spectrophotometers are a convenient and quick way to measure the A260 of the RNA sample. The purity of the RNA sample can be checked by measuring the A260/280 ratio. Ratios within 1.9–2.1 are generally considered pure for RNA; however, note that modified nucleotides can affect these ratios.

Assessing RNA Yield with Qubit™ or RiboGreen™ Assays

Fluorescence-based assays for RNA quantitation is a convenient and sensitive way to measure RNA concentration. If you have access to an Invitrogen™ Qubit™ Fluorometer, the Invitrogen™ Qubit™ RNA BR Assay Kit (Cat. No. Q10210) can be used to measure concentration. If you have a fluorometer or a fluorescence microplate reader, then the Invitrogen™ Quant-iT™ RiboGreen™ RNA Kit (Cat. No. R11490) can be used. Follow the manufacturer's directions if performing either assay.

Agarose Gel Electrophoresis

The RNA can be evaluated by agarose gel electrophoresis to determine its quality and what percentage of the products are full-length. Because RNA forms secondary structures, we recommend using the Thermo Scientific™ RNA Gel Loading Dye (2X) (Cat. No. R0641) to denature the secondary structures and allow the RNA to migrate according to size in a standard TAE agarose gel. **See "RNA Electrophoresis in Agarose Gel"** on page 19 for a protocol on RNA electrophoresis in an agarose gel.

Agilent™ TapeStation™ Systems and RNA Screentape

The RNA can be evaluated on an Agilent™ TapeStation™ system using their RNA ScreenTape assay reagents to determine RNA quality. Follow the manufacturer's instructions for using the TapeStation™ and the RNA ScreenTape assay reagents.

Detection of dsRNA Presence

Several commercially available dsRNA ELISAs are available and can be used to check for the levels of dsRNA in the IVT mRNA. Be aware that the type of base modification used to make the mRNA can affect the sensitivity of these assays.

A dot blot assay to detect dsRNA can be performed; however, it is considered semi-quantitative and is best suited as a qualitative or comparative screening tool rather than a definitive quantification method. **See "Dot blot assay to detect dsRNA"** on page 20 for a dot blot protocol.

Scaled up dsRNA removal procedure

The MEGAclear™ dsRNA removal kit can be scaled up or down linearly with the scale used for IVT. Refer to the following table for guidance.

Component	100 µg IVT scale (1 reaction)[1]	1 mg IVT scale (10 reactions)[1]	10 mg IVT scale (100 reactions)[1]
IVT reaction + DNase	21 µL	0.210 mL	2.1 mL
Water	64 µL	0.640 mL	6.4 mL
10X Binding Buffer	10 µL	0.100 mL	1 mL
dsRNA Binding Protein	5 µL	0.050 mL	0.5 mL
dsRNA Capture Beads	100 µL	1 mL	10 mL[2]

[1] Number of reactions refers to the mMESSAGE mMACHINE™ T7 Transcription kit with CleanCap™ Reagent AG (one 20 µL reaction gives ~100 µg mRNA).

[2] The DynaMag™-15 or DynaMag™-50 Magnets are recommended for large scale reactions.

The RNA can be purified using the MEGAclear™ Transcription Clean-Up Kit, Dynabeads™ RNA Purification Kit, or LiCl Precipitation Solution as described previously. These purification methods also scale up or down linearly. For mRNA synthesis scales 1 mg or greater, Dynabeads™ RNA Purification Kit or LiCl Precipitation Solution is recommended due to ease of use and efficiency.

Note: The loading capacity per MEGAclear™ Transcription Clean-Up column is 500 µg mRNA. Therefore, multiple columns will be needed for scales larger than 500 µg mRNA.

Guidance for alternate use of the kit

This kit is designed for direct application to crude IVT reactions and is not intended to be used solely as a post–RNA purification polishing step. While the kit may be used following RNA purification, performance (including dsRNA removal efficiency and mRNA recovery) may vary and may require additional optimization to achieve desired results. Guidance for post-purification use is provided below.

General Usage Guidelines

1. The suggested ratio of dsRNA Binding Protein to mRNA is 5 µL protein per 100 µg mRNA.

   Note: If mRNA recovery is lower than desired, this ratio may be reduced to improve recovery.

2. The recommended ratio of dsRNA Binding Protein to Capture Beads is 5 µL protein per 100 µL bead slurry.

3. For optimal dsRNA removal and mRNA recovery, an mRNA concentration of 0.5–2 mg/mL is recommended.

4. A downstream mRNA purification step is required following dsRNA removal to remove Binding Buffer components and exchange into a buffer suitable for cell-based or *in vivo* transfection. Purification may be performed using any of the three methods described previously.

A Troubleshooting

Observation	Possible cause	Recommended action
Low Yield of Full-length RNA	Verify that the IVT reaction is functioning correctly. • Confirm that all IVT components have been mixed correctly and that the reaction gives the expected yield prior to performing the dsRNA removal step. This can be done by performing a Qubit™ RNA BR assay to assess the RNA yield in the IVT reaction mixture. Do not measure concentration by reading the absorbance at 260 nm (A260) because this will measure all reaction components, including unincorporated nucleotides and digested DNA. Additional guidance for the IVT step, can be found in the User Guide for the mMESSAGE mMACHINE™ T7 mRNA Kit with CleanCap™ Reagent AG.	If it has been identified that the reaction is of low yield, the following guidelines may help improve the yield: • Confirm the DNA template contains the correct T7 promoter sequence and contains an AG initiation when using the CleanCap™ AG analog. • Confirm the DNA template is of high quality and linearized if using a plasmid. Purification of the DNA template is strongly recommended before performing IVT. • Be sure that the 10X Reaction Buffer in the IVT kit is fresh and has not been exposed to air for long periods of time. Minimize exposure to air as much as possible since this component contains dithiothreitol (DTT) which becomes oxidized when exposed to air. We recommend making aliquots of this component.

Observation	Possible cause	Recommended action
Low Yield of Full-length RNA *(continued)*	Verify if the yield loss is at the dsRNA removal step. • This can be done by performing a Qubit™ assay to assess the RNA yield in the mixture after the dsRNA removal step.	If the yield loss is at this step, the following guidelines may help improve recovery: • Be sure to perform the 5-minute incubation at 37°C described in Step 6 of the dsRNA Removal Procedure as this helps reduce nonspecific interactions between the mRNA and the magnetic beads. • We have seen low recoveries for some mRNA sequences with high GC content (For example: Fluc, GC = 65%). The recoveries can be improved by performing a modified protocol for the dsRNA removal step where the 10X Binding Buffer is reduced by half. For example, for a 100 µL mixture as described in the dsRNA REMOVAL PROCEDURE section, add 5 µL of the 10X Binding Buffer to the mixture instead of 10 µL. • Some templates produce significantly more dsRNA than others, leading to lower mRNA yields after the dsRNA removal step. If this is the case, the template sequence will need to be optimized to improve the recovery. Avoiding repetitive sequences, especially at the end of the template, can help reduce the levels of dsRNA.

Observation	Possible cause	Recommended action
Low Yield of Full-length RNA *(continued)*	If yield loss is not at the IVT or dsRNA removal steps, then it is likely at the RNA purification step. The following guidelines can help improve recovery.	If using the MEGAclear™ Transcription Clean-Up Kit: • Follow the procedure as outlined in the user guide. • The loading capacity per MEGAclear™ column is 500 µg mRNA. Therefore, multiple columns will be needed for scales larger than 500 µg mRNA. • The heat incubation step for elution as described in the user guide can help improve recoveries.
		If using the using the Dynabeads™ RNA Purification Kit: • Follow the procedure as outlined in the user guide. • Be sure to thoroughly mix the sample with the beads before adding the Dynabeads™ RNA Binding Buffer. • Make sure that the beads have fully adhered to the surface of the tube when on the magnet before removing the supernatant. • Perform the washes using freshly made 70% ethanol. • Be sure to mix the beads well with the elution buffer. • A 5 minutes heat incubation step at 37°C with the beads in the elution buffer may improve recovery for some RNAs.
		If using LiCl Precipitation Solution: • Be sure to thoroughly mix the solution after adding LiCl and incubate the solution at -20°C for 30–60 minutes. An overnight incubation may help maximize recovery. • A longer centrifugation step (For example: 20–30 minutes at 16,000–17,000 × g) can help improve recovery. • Perform the wash using freshly made ice cold 70% ethanol. • The pellet may become translucent before it completely dissolves and can be easy to miss. Ensure the pellet is fully dissolved in the resuspension buffer before measuring RNA concentration.

<table>
<tr><th>Observation</th><th>Possible cause</th><th>Recommended action</th></tr>
<tr><td>dsRNA presence still high</td><td>The protocol outlined in this user guide is effective for most transcripts; however, dsRNA formation during IVT can depend strongly on the template sequence. If dsRNA levels remain elevated after dsRNA removal, an additional removal step may further reduce dsRNA content, although this may result in reduced mRNA recovery.

Make sure the mRNA mixture has no biotin for it can interfere with the dsRNA binding protein-bead interaction.</td><td>The following is a suggested protocol for an additional dsRNA removal step at the 100 µg scale. This procedure can be scaled up or down:

<table><tr><th>Component</th><th>Volume (µL)</th></tr><tr><td>Purified mRNA (~1 mg/mL)</td><td>100</td></tr><tr><td>Nuclease-free water</td><td>3</td></tr><tr><td>10X Binding Buffer</td><td>12</td></tr><tr><td>dsRNA Binding Protein</td><td>5</td></tr></table>
Mix well and proceed to Step 2 of the dsRNA REMOVAL PROCEDURE section.

Note: We have observed that the additional dsRNA removal step helps mRNAs with no modified nucleotides (eg: unmodified mRNA).</td></tr>
<tr><td>RNA transcript smearing on gel</td><td>Transcript smearing on a gel is usually indicative of RNase contamination. Be sure that all steps are performed under RNase-free conditions with RNase-free consumables and reagents.</td><td>Use RNase-free pipette tips and tubes and nuclease-free water. It is also important that the gel tank is used only with RNase-free reagents. The tank can be cleaned with 0.1N NaOH or RnaseZap (Cat. No. AM9780).</td></tr>
<tr><td>Magnetic beads not binding to the magnet</td><td>This is only an issue if not using the recommended magnetic stands. Using any of the stands listed on “Required materials not supplied” on page 7 will resolve the issue.</td><td>If using a different magnet source, the incubation time may need to be extended until the solution clears and a tight pellet is formed at the magnet.</td></tr>
</table>

B

Additional procedures

RNA Electrophoresis in Agarose Gel

RNA can be analyzed in a standard TAE agarose gel by following the steps below:

1. Pour an agarose gel of the appropriate percentage for the size of the RNA transcript. We recommend 1% agarose for transcripts >500 bases and 2.5% for those <500 bases.
   - For 100 mL of gel, add 100 mL 1X TAE buffer to 1 or 2.5 g of agarose.
   - Heat the solution in a microwave oven until bubbles appear.
   - Remove the flask carefully, and swirl gently to resuspend any agarose particles.

CAUTION! Microwaved solution may become superheated and foam over when agitated.

   - Reheat the solution until the solution comes to a boil, and all the agarose particles are dissolved.
   - Remove the flask carefully and swirl gently to mix the solution. Allow it to cool to 50–60°C.
   - Add 5 µL of SYBR Safe DNA Gel Stain (Cat. No. S33102) and swirl gently to mix the solution.
   - Pour the gel and allow it to set.

2. Prepare the RNA samples.
   - Add an equal volume of 2X RNA Loading Dye (Cat. No. R0641) to the RNA sample and mix well. We recommend a final RNA concentration of 10–15 ng/µL
   - Heat the mixture at 70°C for 10 minutes.
   - Immediately chill on ice for 5 minutes.
   - Spin down prior to loading on a gel.

3. Load the samples and run the gel in 1X TAE buffer at ~5 volts/cm until the bromophenol blue dye has migrated ~3/4 of the gel. Examine the gel on an appropriate instrument (For example: an iBright™ CL1500 Imaging System).

Dot blot assay to detect dsRNA

Required materials not supplied:

1. IVT mRNA sample at 50 ng/µL.
2. Novex Nylon+ Membrane, 0.45 um pore size (Cat. No. LC2003).
3. WesternBreeze Blocker/Diluent – Part A & B (Cat. No. WB7050).
4. WesternBreeze Wash Solution (Cat. No. WB7003).
5. Double-stranded RNA Monoclonal Antibody (J2) (Cat. No. 10010200-200UG).
6. Secondary Antibody Solution Alk-Phos. Conjugated (Anti-Mouse) (Cat. No. WP20006).
7. Novex AP Chemiluminescent Substrate (CDP-Star) (Cat. No. WP20002).
8. Instrument for visualizing probed membrane (For example: iBright™ CL1500 Imaging System).
9. Deionized (diH_2O) or ultra-pure water (For example: Cat. No. 10977015).

Protocol

1. Add 2 µL of the IVT mRNA (50 ng/µL) to the dry membrane. Be careful not to touch the membrane with the pipette tip. This means each dot corresponds to 100 ng IVT mRNA.

   Note: A positive dsRNA control is recommended. Several dsRNA substrates are commercially available such as 142 bp dsRNA control from Jena Bioscience. We suggest to add 2 µL of 25–12.5 ng/µL of the dsRNA control to the membrane so that the dot corresponds to 50–25 ng dsRNA.

2. Allow membrane to dry at room temperature >2 hours or overnight.
3. Prepare the blocking solution: 5 mL H_2O + 2 mL Diluent A + 3 mL Diluent B.
4. Add blocking solution to membrane. Incubate at room temperature on a shaker for >2 hours.
5. Rinse membrane twice with diH_2O.
6. Prepare the primary solution: 7 mL H_2O + 2 mL Diluent A + 1 mL Diluent B. Add 2 µL of the J2 antibody (1:5000 dilution).
7. Add primary solution to membrane. Incubate at room temperature overnight on a shaker.
8. Prepare the wash solution: 150 mL H_2O + 10 mL Wash Solution.
9. Perform 5 minutes washes five times. Add 20 mL diluted wash solution per wash.

10. Prepare the secondary solution: 7 mL H_2O + 2 mL Diluent A + 1 mL Diluent B. Add 1 µL of the secondary antibody (1:10,000 dilution).

11. Add secondary solution to membrane. Incubate at room temperature at least 1 hour.

12. Perform 5 minutes washes five times. Add 20 mL diluted wash solution per wash.

13. Rinse membrane twice with diH_2O.

14. Add 1–1.5 mL of AP substrate so that it covers the entire membrane.

15. Incubate at room temperature 5 minutes.

16. Visualize the membrane (For example: iBright™ using the Chemi Blots setting).

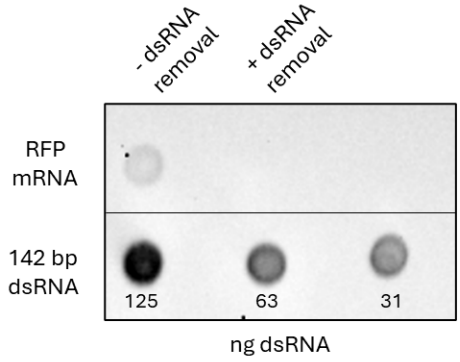

Figure 2 Example dot blot analysis of dsRNA removal from IVT RFP mRNA. The mRNA was subjected to the dsRNA removal process, and residual dsRNA was assessed by dot blot in comparison with a non-treated control sample. No detectable dsRNA signal was observed after dsRNA removal.

C Related products

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.

Item	Cat. No.
mMessage mMACHINE™ Transcription kit with CleanCap™ AG	A57620-25
MEGAscript™ T7 Transcription Kit Plus	A57622-25
TheraPure™ GMP N1-Methylpseudo-UTP, 100 mM sodium solution	R0491SKB007
DynaMag™–2 Magnet	12321D
MEGAclear™ Transcription Clean-up Kit	AM1908
Dynabeads™ RNA Purification Kit	65030D
LiCl Precipitation Solution (7.5 M)	AM9480
KingFisher™ Flex Purification System, KingFisher™ with 96 PCR head	5400610
THE RNA Storage Solution	AM7001
Qubit™ RNA BR Assay Kit	Q10210
Quant-iT™ RiboGreen™ RNA Kit	R11490
RNA Gel Loading Dye (2X)	R0641
RNase*Zap*™ RNase Decontamination Solution	AM9780
RiboRuler™ Low Range RNA Ladder	SM1831
RiboRuler™ High Range RNA Ladder	SM1821
Lipofectamine™ MessengerMAX™ Transfection Reagent	LMRNA003
Vivofectamine™ VF233 Liver LNP Composition in Ethanol	VF232LVCE
Vivofectamine™ VF233 IM LNP Composition in Ethanol	VF233IMCE
UltraPure™ Agarose	16500100
Owl™ A2 Large Gel Systems	A2-BP

D

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  cdc.gov/labs/bmbl
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  who.int/publications/i/item/9789240011311

Documentation and support

ISO certification

Manufactured by Thermo Fisher Scientific Baltics UAB, in compliance with ISO 9001 and ISO 13485 certified quality management system.

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.