PRODUCT INFORMATION

Thermo Scientific
MagJET mRNA Enrichment Kit

#K2811, #K2812

Lot 00000000
Exp. 00.0000

Read Storage information (p. 4) upon receipt and store kit components appropriately!

www.thermoscientific.com/onebio

CERTIFICATE OF ANALYSIS

Thermo Scientific™ MagJET™ mRNA Enrichment Kit was qualified by isolating mRNA from 30 µg of total HeLa RNA following the protocol outlined in the manual. The quality of isolated mRNA is determined by measuring rRNA contamination using RT-qPCR. The amount of rRNA is <0.5% from starting material.

Quality authorized by:  Jurgita Žilinskienė

Rev.3

CONTENTS page

COMPONENTS OF THE KIT

MagJET mRNA Enrichment Kit	#K2811 10 preps	#K2812 96 preps
Hybridization Buffer for MagJET mRNA Enrichment Kit	4.5 mL	45 mL
MagJET oligo (dT) Beads	0.55 mL	5.5 mL
Wash Buffer for MagJET mRNA Enrichment Kit	22 mL	220 mL
Water, nuclease-free	2 × 1.25 mL	30 mL

STORAGE

MagJET mRNA Enrichment Kit should be stored at 4 °C.

DESCRIPTION

The MagJET mRNA Enrichment Kit is designed for fast and efficient purification of mRNA from total RNA samples. The oligo (dT) beads bind poly A+ specifically and with high capacity, which ensures maximum extraction of mRNA from a range of starting material amount.

The kit utilizes paramagnetic bead technology enabling high yields and robust performance. High binding capacity, uniform particle size, and rapid magnetic response of MagJET oligo (dT) beads makes the technology ideal for high throughput automated nucleic acid purification, as well as for manual nucleic acid purification by low sample throughput users.

MagJET mRNA Enrichment Kit results in the highest intact full length RNA transcripts and low rRNA contamination.The resulting high quality purified mRNA is free of proteins, nucleases and other contaminants or inhibitors, can be used in a wide range of downstream applications, such as NGS, RT-PCR, RT-qPCR and other enzymatic reactions.

PRINCIPLE

The MagJET mRNA Enrichment Kit uses the highly efficient magnetic particle based-technology of MagJET oligo (dT) beads for nucleic acid purification. The whole nucleic acid isolation process combines simple steps of mRNA binding to the magnetic beads, rRNA removal, washing and elution.

Purification protocols optimized for automated KingFisher instruments utilize a high throughput magnetic bead transfer technique, where magnetic beads are transferred through different reagent plates containing binding, washing and elution reagents. This enables high throughput nucleic acid purification and eliminates multiple pipetting steps.

Alternatively, a protocol is provided where instead of magnetic particles, buffers and other reagents are transferred in each of the protocol steps, while magnetic beads remain captured on the wall of the tube using a magnetic rack. This allows the kit to be used in various throughput applications using a magnetic rack and manual or automated pipetting equipment.

IMPORTANT NOTES

Check all solutions for any salt precipitation before each use. Re-dissolve any precipitate by warming the solution at 50-60 °C, and then equilibrate to room temperature (15-25 °C).

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Pipettes and RNase-free pipette tips
- Thermomixer (optional)
- Vortex
- Microcentrifuge
- 1.5 mL tubes, RNase-free
- Disposable gloves
- Automatic magnetic particle processor and consumables *or*
- Magnetic rack

AVOIDING RIBONUCLEASE CONTAMINATION

RNA purity and integrity is essential for downstream applications. RNA can be degraded by RNases, which are a highly stable contaminant found in any laboratory environment. Care must be taken not to introduce RNases into the RNA preparation.

General recommendations to avoid RNase contamination:

- Wear gloves when handling reagents and RNA samples, as skin is a common source of RNases. Change gloves frequently.
- Use sterile, disposable RNase-free pipette tips.
- Use appropriate reagents to remove RNase contamination from non-disposable items such as pipettes, centrifuges and work surfaces.
- Keep all kit components tightly sealed when not in use. After use close bottles immediately.

STARTING MATERIAL HANDLING AND STORAGE

- Make sure that the starting material (total RNA) is of good quality and integrity to achieve good mRNA yields.
- Always store RNA samples at -70 °C and avoid multiple freeze-thaw cycles.

PROTOCOL SELECTION GUIDE

The following selection guide summarizes available protocols depending on the throughput and sample processing type. The MagJET mRNA Enrichment Kit provides optimized protocols for mRNA purification from total RNA samples starting from 5 µg to 100 µg. The kit is compatible with automated and manual processing.
Automation protocols are optimized for KingFisher Flex and KingFisher Duo instruments.

Note: Transfer the mRNA_Flex protocol file to the KingFisher Flex or mRNA_Duo protocol file to the KingFisher Duo instrument before first use. The instructions for transferring the protocol can be found in Chapter 4: "Using the software" in the BindIt Software for KingFisher Instruments version 3.2 User Manual. The protocol files for MagJET mRNA Enrichment Kit can be found on the product web page on www.thermoscientific.com/onebio

Protocol selection guide:

Sample type	Sample quantity	Throughput per run	KingFisher Flex Instrument	KingFisher Duo Instrument	Manual processing	MagJET purification protocol	Page
Total RNA	5-100 µg	96	●	-	-	Protocol A	page 7
		12	-	●	-	Protocol B	page 9
		variable	-	-	●	Protocol C	page 11

Protocol A. Instructions for mRNA purification from total RNA using KingFisher Flex 96 and Microtiter deep well 96 plates

Note: If the initial amount of total RNA is less than 25 µg, prepare the sample in 50 µL total volume and resuspend magnetic beads in step 3 with corresponding amount of Hybridization Buffer (1:1).

Transfer the mRNA_Flex protocol file to the KingFisher Flex as described on page 6.

1. Obtain six empty Thermo Scientific Microtiter deep well 96 plates (Cat. Nr 95040460) and two empty Thermo Scientific KingFisher 96 KF plates (Cat. Nr 97002540).
2. Prepare the total RNA in 100 µL RNase-free water (or prepare in 50 µL if sample is less than 25 µg). Heat the sample at 65 °C for 5 min. Place it on ice immediately.
3. Wash 50 µL of MagJET oligo (dT) Beads with 100 µL of Hybridization Buffer two times and then resuspend the beads in 100 µL of Hybridization Buffer (resuspend in 50 µL of Hybridization Buffer if the total RNA sample was prepared in 50 µL). During each wash, mix by pipeting and then collect the particles using a magnetic separator stand, in accordance to the manufacturer's instructions. Whenever the particles are collected using a magnetic separator, leave the tube in the magnetic stand while removing the supernatant.

Note. Resuspend Magnetic beads well by vortexing before use.

4. Add the sample and resuspended magnetic beads to the Sample plate (Microtiter deep well 96 plate).
5. Prepare the plates as follows:

Plate number	Plate type	Plate name	Content	Volume per well
2	Microtiter deep well 96 plate	Wash 1	Wash Buffer	500 µL
3		Wash 2	Wash Buffer	500 µL
4		Elution 1	Water, nuclease-free	100 µL
5		Wash 3	Wash Buffer	500 µL
6		Wash 4	Wash Buffer	500 µL
7	KingFisher Flex 96 KF plate	Elution 2	Water, nuclease-free	50 µL
8		Tip Plate	---	---

6. Add the following reagents to the **Sample** plate.

Plate number	Plate type	Plate name	Content	Volume per well
1	Microtiter deep well 96 plate	Sample	Sample	100 µL (or 50 µL)
			Magnetic beads in Hybridization Buffer	100 µL (or 50 µL)

7. Place a Thermo Scientific KingFisher Flex 96 tip comb for deep well magnets (Cat. Nr 97002534) on a **Tip Plate** (KingFisher Flex 96 KF plate).
8. Start the **mRNA_Flex** protocol on the KingFisher Flex 96 and load the plates according to the KingFisher display. After all the plates have been loaded into the instrument, the protocol will begin.
9. When the KingFisher Flex pauses at the dispense step after the first elution, (approximately 20 minutes after starting the run), remove the **Elution 1** plate from the instrument and add 100 µL of the **Hybridization Buffer** per well to the **Elution 1** plate to rebind the mRNA.

Plate number	Plate type	Plate name	Content	Volume per well
4	Microtiter deep well 96 plate	Elution 1	Hybridization Buffer	100 µL

10. Place the plate back into the instrument and press **Start**. After the pause, the protocol will continue to the end.
11. When the protocol is completed, remove the plates according to the instructions on the KingFisher Flex display and turn off the instrument. Transfer the eluate (**Elution 2**, which contains the purified mRNA) to a new, sterile tube and close immediately. The purified mRNA is ready for use in downstream applications. Keep the purified RNA on ice for immediate use, or store at -70 °C.

Protocol B. Instructions for mRNA purification from total RNA using KingFisher Duo and Microtiter deep well 96 plates

Note: If the initial amount of total RNA is less than 25 µg, prepare the sample in 50 µL of water and resuspend magnetic beads in step 3 with corresponding amount of Hybridization Buffer (1:1).

Transfer the mRNA_Duo protocol file to the KingFisher Flex as described on page 6.

1. Obtain one empty Thermo Scientific Microtiter deep well 96 plate (Cat. Nr 95040460) and one Thermo Scientific KingFisher Duo elution strip (Cat. Nr 97003520)
2. Prepare the total RNA in 100 µL RNase-free water (or prepare in 50 µL if sample is less than 25 µg). Heat the sample at 65 °C for 5 min. Place it on ice immediately.
3. Wash 50 µL of MagJET oligo (dT) Beads with 100 µL of Hybridization Buffer two times and then resuspend the beads in 100 µL of Hybridization Buffer (resuspend in 50 µL of Hybridization Buffer if the total RNA sample was prepared in 50 µL). During each wash, mix by pipeting and then collect the particles using a magnetic separator stand, in accordance to the manufacturer's instructions. Whenever the particles are collected using a magnetic separator, leave the tube in the magnetic stand while removing the supernatant.

Note: Resuspend magnetic beads well by vortexing before use.

4. Add the sample and resuspended magnetic beads to the Sample plate (Microtiter deep well 96 plate) row F.
5. Prepare the Sample plate (Microtiter deep well 96 plate) according to the instructions below.

Add the following reagents to the indicated rows. Note that row H is reserved for the tip comb and should be left empty. Note that row G is left empty.

Plate name and type	Row	Row name	Content	Volume per well
Sample plate Microtiter deep well 96 plate	A	Elution 1	Water, nuclease-free	100 µL
	B	Wash 4	Wash Buffer	500 µL
	C	Wash 3	Wash Buffer	500 µL
	D	Wash 2	Wash Buffer	500 µL
	E	Wash 1	Wash Buffer	500 µL
	F	Sample	Sample	100 µL (or 50 µL)
			Magnetic Beads resuspended in Hybridization Buffer	100 µL (or 50 µL)
	G	Empty	Empty	Empty
	H	Tip Comb	12-tip comb	---

6. Fill the KingFisher Duo Elution Strip as follows:

Elution strip	Content	Reagent volume per well
KingFisher Duo elution strip	Water, nuclease free	50 µL

7. Place a Thermo Scientific KingFisher Duo 12-tip comb (Cat. Nr 97003500) into row H of the Sample plate.

8. Switch on the KingFisher Duo. Start the mRNA_Duo protocol and load the plate and Elution Strip according to the KingFisher display. Ensure that the elution strip is placed in the correct direction into the elution block and that the perforated end is facing towards the user. After all plates have been loaded the program will start.

9. When the KingFisher Duo pauses at the dispense step after the Elution 1 step (approximately 20 minutes after starting the run), remove the Sample plate from the instrument and add 100 µL Hybridization Buffer per well to row A to rebind the mRNA.

Row	Row name	Content	Volume per well
A	Elution 1	Hybridization Buffer	100 µL

10. Place the plate back into the instrument and press OK. After the pause, the protocol will continue to the end.

11. After the run is completed, remove the Sample plate and Elution Strip according to the instructions on the KingFisher Duo display and turn off the instrument. Transfer the eluate (Elution Strip, which contains the purified mRNA) to a new, sterile tube and close immediately. The purified RNA is ready for use in downstream applications. Keep purified RNA on ice for immediate use, or store at -70 °C.

Protocol C. Instructions for manual mRNA purification from total RNA.

The following protocol is based on transfer of liquids by pipetting through different purification steps rather than magnetic bead transfer as in KingFisher automated protocols. This allows the kit to be used in various throughput applications using a magnetic rack and manual or automated pipetting equipment. Protocols for the other automated pipetting platforms should be optimized for each platform and sample used. To enable protocol optimization, all buffers are available to purchase separately.

Note: If the initial amount of total RNA is less than 25 µg, prepare the sample in 50 µL of water and resuspend magnetic beads in step 5 with corresponding amount of Hybridization Buffer (1:1).

1. Prepare the total RNA in 100 µL of RNase-free water (or prepare in 50 µL if sample is less than 25 µg).

2. Heat the total RNA solution at 65 °C for 5 minutes, and then transfer the sample to ice immediately.

3. Vortex the vial of MagJET oligo (dT) Beads to ensure that they are in homogenous suspension. Transfer 50 µL of the bead suspension to an RNase-free microcentrifuge tube.

4. Wash the particles twice with 100 µL of Hybridization Buffer. During each wash, mix by pipeting and then collect the particles using a magnetic separator stand, in accordance to the manufacturer's instructions. Whenever the particles are collected using the magnetic separator, leave the tube in the magnetic stand while removing the supernatant.

5. Resuspend the washed particles in 100 µL of Hybridization Buffer by repeated pipeting (resuspend in 50 µL of Hybridization Buffer if the total RNA sample was prepared in 50 µL).

6. Add total RNA solution to the resuspended magnetic particles. Mix by repeating pipeting.

7. Incubate the mixture at room temperature with gentle agitation for 5 minutes to allow hybridization of the mRNA to the magnetic beads.

8. Collect the magnetic particles complexed with mRNA using magnetic separator stand.

9. Remove the supernatant with a pipette while the tube is still in the magnetic stand.

10. Wash magnetic particles three times using 100 µL of Wash Buffer for each wash. Take the tube out from the magnetic stand, add Wash Buffer, mix thoroughly by pipeting and then recollect the beads using the magnetic separator stand. Be sure to remove all of the Wash Buffer when completing the final wash.

11. Remove the tube from the magnetic stand and then add 50 µL of nuclease-free water to the beads. Incubate the tube with gentle agitation for 2 minutes at 60 °C and return the sample to room temperature.

12. Add 50 µL of Hybridization Buffer to each sample to rebind mRNA to the beads. Gently pipette the entire volume up and down 6 times to mix thoroughly and incubate at room temperature for 5 minutes.

13. Collect the magnetic particles complexed with mRNA using magnetic separator stand.

14. Remove and discard all of the supernatant from each tube. Take care not to disturb the beads.

15. Wash the beads twice with 100 µL of Wash Buffer. Take the tube out from the magnetic stand, add Wash Buffer, mix thoroughly by pipeting and then recollect the beads using the magnetic separator stand. Be sure to remove all of the Wash Buffer when completing the final wash.

16. Elute the mRNA from the beads by adding 50 µL of nuclease-free water and incubating the sample at 60 °C for 2 minutes. Immediately place the tubes on the magnetic rack after the 2 minutes.

17. Collect the purified mRNA by transferring the elution to a clean nuclease-free tube and place on ice.

TROUBLESHOOTING

Problem	Possible Cause and Solution
Low RNA yield	Incomplete re-suspension of magnetic particles. Fully resuspend the magnetic particles by vortexing before use. Make sure Hybridization Buffer is added to the sample at a 1:1 ratio. *Manual protocol only:* Loss of magnetic beads during manual purification Be careful not remove the magnetic beads during purification using manual protocol. Magnetic beads not fully dispersed during elution step Make sure the magnetic beads are fully dispersed in nuclease-free water during elution step.
Degraded RNA	Inappropriate handling of starting material. Check total RNA sample for RNase contamination. Analyze sample by agarose gel electrophoresis. RNase contamination can be determined by loss or smear of 18S and 28S rRNA bands. RNase contamination. To avoid RNase contamination, wear gloves during all procedures and change gloves frequently. Use sterile, disposable RNase-free pipette tips. Remove RNase contamination from non-disposable items and work surfaces.
Low purity	Insufficient washing Insufficient washing causes impurities in the elution step with nuclease free water. Ensure that volumes of the Wash Buffer are as indicated in the protocol. *Manual protocol only:* Wash Buffer not fully removed during wash steps Make sure that all wash solution is removed during wash steps.
Genomic DNA contamination	Perform DNase I digestion of total-RNA samples contaminated with genomic DNA before poly(A)-RNA purification.
Magnetic particles in the purified RNA	Carryover of the MagJET oligo (dT) Beads to the elution step may affect the A_{260}/A_{280} ratio, however the magnetic beads in the eluted RNA will not affect downstream applications. To remove carryover magnetic particles, place eluted sample in the magnetic rack once again. Carefully transfer eluate to a clean, sterile microcentrifuge tube. Magnetic beads that occasionally remain attached to the tip combs at the end of the process do not affect the mRNA yield, as the RNA has already been released into the nuclease free water.

SAFETY INFORMATION

In accordance with European Directives 67/548/EC and 1999/45/EC and Regulations 1907/2006/EC and 1272/2008/EC the product does not classified as hazardous.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

© 2013 Thermo Fisher Scientific, Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific, Inc. and its subsidiaries.