invitrogen™

mRNA Catcher™ *PLUS*

Catalog No.	Format:
K1570-02	1 x 96-well plate
K1570-03	10 x 96-well plates

Contents and Storage

The components included with the mRNA Catcher™ *PLUS* are listed below. Upon receipt store all components as described in the table below. Sufficient reagents are included to perform 1 x 96 mRNA isolations (K157002) or 10 x 96 mRNA isolations (K157003).

Components	K1570-02	K1570-03	Storage
mRNA Catcher™ *PLUS* plate	1 x 96-well plate	10 x 96-well plates	4°C
2X Lysis Buffer (L20)	4 ml	40 ml	Room temperature
Wash Buffer (W15)	30 ml	2 x 150 ml	Room temperature
Dilution Buffer (DL1)	4 ml	40 ml	Room temperature
Elution Buffer (E3; DEPC-treated RNase-free water)	8 ml	80 ml	Room temperature
0.5 M DTT	100 µl	1 ml	4°C

Description

The mRNA Catcher™ *PLUS* 96-well plate and reagents allow fast, high-throughput isolation of polyA-tailed mRNA from small sample sizes of 100 to 10^6 cells per well. The mRNA Catcher™ *PLUS* plate is designed to isolate mRNA from cells, animal tissues, whole blood, and total RNA with negligible genomic DNA contamination and is compatible with most automated liquid handling workstations. Well-to-well and plate-to-plate variability is low ensuring high reproducibility. The isolated mRNA is compatible for RT-PCR, real time quantitative RT-PCR (qPCR), and cDNA synthesis and microarray studies if combined with RNA amplification.

The mRNA Catcher™ *PLUS* 96-well plate is coated with immobilized single-stranded poly(dT) oligonucleotide containing LNA™ (Locked Nucleic Acid) nucleotides and allows for isolating of more than 80 ng mRNA per well, depending on the sample. The LNA™ is a novel type of nucleic acid that contains a 2′-O, 4′-C methylene bridge. This configuration locks the sugar backbone in 3′-endo conformation resulting in an increase in T_m (melting temperature). The incorporation of LNA™ into the oligonucleotide probe increases the hybridization efficiency and specificity of the mRNA from samples without the need for increasing the amount or concentration of the sample. The use of mRNA Catcher™ *PLUS* plate with LNA™ oligonucleotides provides higher yield and purity of the isolated mRNA as compared to using plates with standard oligonucleotides.

System Overview

The mRNA Catcher™ *PLUS* is a solid phase high-throughput mRNA isolation system in 96-well format. Cells, tissue, and blood samples are lysed using the Lysis Buffer containing chaotropes that protect the RNA from endogenous RNases. The mRNA from lysates is hybridized to a single stranded poly (dT) oligonucleotide containing LNA™ immobilized on the mRNA Catcher™ plate. The plate is washed with Wash Buffer to remove unbound material. The purified mRNA bound to the plate can be directly reverse transcribed to cDNA in the well or the mRNA can be eluted in Elution Buffer and is suitable for any downstream application including qRT-PCR.

Accessory Products

The following products and a wide variety of RT-PCR products available from Invitrogen may be used with the mRNA Catcher™ *PLUS* 96-well plates. For more information, visit our website at www.invitrogen.com.

Product	Quantity	Catalog no
mRNA Catcher™ *PLUS* Buffers	960 rxns	K157004
RNase AWAY®	250 ml	10328-011
SuperScript™ III First-Strand Synthesis System for RT-PCR	50 rxns	18080-051
Platinum® PCR Supermix	100 rxns	11306-016
LUX™ Fluorogenic Primer Set, FAM-labeled	50 nmol or 200 nmol	Design and order LUX™ Primer Sets at www.invitrogen.com/lux
LUX™ Fluorogenic Primer Set, JOE-labeled	50 nmol or 200 nmol	
PureLink™ Foil Tape	50 tapes	12261-012
Platinum® Quantitative PCR Supermix-UDG (with ROX)	500 rxns	11730-025 (11743-500)

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General Guidelines

- Wear a laboratory coat, disposable gloves, and eye protection when handling reagents and plate
- Always use proper aseptic techniques when working with RNA and use only sterile, RNase-free tips to prevent RNase contamination
- After elution of mRNA from the well, unused wells of plate can be saved for later RNA isolations by returning covered plate to the re-sealable pouch and storing the plate at 4°C for up to 6 months.

Materials Needed

- RNase-free microcentrifuge tubes and RNase-free tips
- Microcentrifuge capable of centrifuging at a speed of 13,000-15,000 x g
- Heat block or thermocycler, set to 68°C that can accommodate a 96-well plate
- Phosphate Buffered Saline (PBS, GIBCO® cat. no. 10010-023)

Before Starting

- Bring all reagents to room temperature
- Mix 2X Lysis Buffer well. If the buffer contains precipitates, incubate at 37°C to solve the precipitates
- Prepare **fresh** 2X Lysis Buffer with DTT by adding 25 µl 0.5 M DTT to 975 µl 2X Lysis Buffer (final DTT concentration is 5 mM) to prepare 1 ml of 1X Lysis Buffer with DTT. Mix well and use for preparing the cell lysate. Depending on the amount and type of samples you are preparing, you can scale-up the volumes accordingly.

  Note: If you use the entire kit for preparing mRNA from Adherent Cells using *Protocol B* or from total RNA, transfer the entire amount of Dilution Buffer (DL1) into the bottle containing 2X Lysis Buffer with DTT to prepare 1X Lysis Buffer with DTT.

Preparing Lysates

Suspension Cells

Grow your cells in a flask or in 24-, 48- or, 96-well cell culture plates. Use 100 to 10^6 cells per well of the mRNA Catcher™ *PLUS* plate. If you are using 24-, 48- or, 96-well cell culture plates, you may proceed directly to Step 2.

1. If your cells are grown in a flask, transfer suspension cells from the flask into RNase free tubes.
2. Centrifuge to pellet cells and remove the media.
3. Resuspend the cell pellet into an appropriate volume of PBS to obtain 3 x 10^3 to 3 x 10^7 cells/ml.
4. Transfer 30-40 µl cell suspension containing 100 to 10^6 cells into each well of the mRNA Catcher™ plate.
5. Add equal volume of 2X Lysis Buffer with DTT to each well. Mix well by pipetting up and down.
   Note: To completely cover the oligonucleotide immobilized onto the well, use up to 80 µl cell lysate volume for each well.
6. Proceed to **Isolating mRNA**, next page.

Adherent cells

Grow your cells in a flask or in 24-, 48- or, 96-well cell culture plates. Use 100 to 10^6 cells per well of the mRNA Catcher *PLUS*™ plate. Harvest adherent cells using trypsin (*Protocol A*) or Lysis Buffer (*Protocol B*) as described below.

Protocol A: *Trypsin Treatment*

1. Aspirate the cell media from wells or flask and perform trypsinization.
2. Wash the trypsinized cells once with PBS. Aspirate the PBS completely.
3. Resuspend the cells in an appropriate volume of PBS to obtain 3 x 10^3 to 3 x 10^7 cells/ml.
4. Transfer 30-40 µl cell suspension containing 100 to 10^6 cells into each well of the mRNA Catcher™ plate.
5. Add the same volume of 2X Lysis Buffer with DTT to each well. Mix well by pipetting up and down.
   Note: To completely cover the oligonucleotides immobilized onto the well, use up to 80 µl cell lysate with 2X Lysis Buffer with DTT volume for each well.
6. Proceed to **Isolating mRNA**, next page.

Protocol B: *Lysis Buffer Treatment*

1. Mix 2X Lysis Buffer with DTT with equal volume of 1X Dilution Buffer to obtain 1X Lysis Buffer with DTT.
2. Aspirate the cell media from wells or flask.
3. Wash cells once with 100 µl PBS. Aspirate the PBS completely.
4. Add 85 µl 1X Lysis Buffer with DTT to each cell sample.
5. Incubate for 3-6 minutes at room temperature to lyse the cells.
6. Transfer 80 µl cell lysate to each well of the mRNA Catcher *PLUS*™ plate.
7. Proceed to **Isolating mRNA**, next page.

Human Whole Blood (fresh, frozen, containing anticoagulants such as EDTA or Heparin)

1. For each sample, transfer 40 µl 2X Lysis Buffer with DTT into a separate well of mRNA Catcher™ *PLUS* plate.
2. Add 40 µl whole blood to each well.
3. Mix well by pipetting up and down.
4. Proceed to **Isolating mRNA,** next page.

Preparing Lysates, continued

Total RNA

1. Mix 2X Lysis Buffer with DTT with an equal volume of 1X Dilution Buffer (DL1) to obtain 1X Lysis Buffer with DTT.
2. Mix 80 µl of 1X Lysis Buffer with 100 ng to 100 ug of total RNA.
1. Transfer 80 µl of the RNA solution to each well of the mRNA Catcher™ plate.
2. Proceed to **Isolating mRNA**, below.

Animal Tissues (fresh or frozen)

Choose between the two protocols below for qualitative studies (*Tissue Protocol A*) and quantitative studies (*Tissue Protocol B*)

Note: If you process more than 4 mg tissue per well, scale-up the amount of 2X Lysis Buffer with DTT and Wash Buffer accordingly. Since the amount of buffers provided in the kit is sufficient to process 96 samples of 4mg tissue, you may have to compensate for the increased volumes of buffers you are using when processing larger tissue by reducing the number of samples accordingly.

Tissue Protocol A - Procedure for Qualitative Studies

1. Dissect tissue of interest using RNase-free, sterile equipment and transfer 4-40 mg of dissected tissue directly into the well of mRNA Catcher™ *PLUS* plate.
2. Add 10 µl (per mg of dissected tissue) of 2X Lysis Buffer with DTT to the well. Ensure that tissue is completely submerged in buffer.
3. Incubate for 90 minutes at room temperature and discard remaining tissue pieces and buffer.
4. Proceed to **Washing Step**, below.

Tissue Protocol B - Procedure for Quantitative Studies

1. Dissect tissue of interest using RNase-free, sterile equipment and transfer 4-40 mg of dissected tissue into a 1.5 ml RNase-free microcentrifuge tube.
2. Add 10 µl (per mg of dissected tissue) of 2X Lysis Buffer with DTT into the tube. Ensure that all tissue is submerged in buffer.
3. Grind tissue using an RNase-free pestle tissue grinder. Use a different pestle for each tissue sample.
4. After homogenization, spin the tube at 13,000-15,000 x g for 2 minutes at room temperature using a microcentrifuge.
5. Transfer up to 80 µl of the supernatant containing the RNA into each well of mRNA Catcher™ *PLUS* plate.
   Optional: Transfer the supernatant into a clean RNase-free microcentrifuge tube. If the supernatant still contains residues of tissue, repeat Steps 4 and 5.
6. Proceed to **Isolating mRNA**.

Isolating mRNA

Use the prepared lysates to purify the mRNA in the mRNA Catcher™ *PLUS* plate. You can elute the purified mRNA after purification or directly use the purified mRNA attached to the plate for immediate downstream applications by omitting the elution step and proceeding to the Downstream Application protocol, on the next page.

Hybridization Step

1. Cover the mRNA Catcher™ *PLUS* plate containing samples with adhesive foil plate cover (see page 1 for ordering information).
2. Incubate the plate for 45-60 minutes at room temperature for RNA hybridization.
3. Proceed to **Washing Step**, below.

Washing Step

1. Aspirate the lysates from wells. Be sure not to scrape the well sides during aspiration.
2. Add 100 µl Wash Buffer (W15) to the wells
3. Incubate the plate for 1 minute at room temperature.
4. Aspirate the Wash Buffer. Repeat washing twice to obtain a total of 3 washes.
5. After the final wash, completely aspirate any remaining Wash Buffer (W15).

   Note: If performing the **Downstream Application** directly in the mRNA Catcher™ *PLUS* plate with the attached mRNA prepare the RT-Solution Mix (next page) BEFORE removing the Wash Buffer to avoid drying of the mRNA.

6. Proceed to **Eluting mRNA**, below. Alternatively, proceed to **Performing cDNA Synthesis on mRNA attached to the mRNA Catcher™ *PLUS* plate**, next page.

   Note: If performing the cDNA synthesis reaction directly in the mRNA Catcher™ plate, immediately proceed to **Downstream Applications** without performing the elution step.

Elution Step

1. Add 80 µl Elution Buffer (E3) into wells of the mRNA Catcher™ *PLUS* plate.
2. Cover the plate with foil plate cover and incubate the plate at 68°C for 5 minutes. Immediately cool the plate to 4°C using a thermocycler with heated lids to avoid condensation. If a thermocycler is not available, transfer the plate on ice.
3. Transfer the eluted mRNA from the wells to RNase-free microcentrifuge tubes or sealable microtiter plates.
4. Store the mRNA at -80°C until use.

Downstream Applications

The mRNA isolated using the mRNA Catcher™ *PLUS* plate is suitable for use in RT-PCR, reverse transcription, and qPCR reactions without the need to perform any additional steps. A protocol to perform cDNA synthesis directly in the mRNA Catcher™ *PLUS* plate without prior elution of the mRNA is described below.

Performing cDNA Synthesis from mRNA Attached to the mRNA Catcher™ *PLUS* Plate Using Exogenous Primers

The cDNA synthesis reaction is performed using exogenous random hexamer or oligo(dT)$_{12-18}$ primers. Instructions for cDNA synthesis using the **SuperScript™ III First-Strand Synthesis Systems for RT-PCR, available from Invitrogen**, are provided below. For details, refer to the manual supplied with the RT-PCR kits or download the manuals from our website at www.invitrogen.com.

Note: If you use other products for cDNA synthesis, refer to the manuals provided with the products and replace the sample volume with RNase-free water when preparing the RT-solution.

1. Prepare the RT-Solution Mix using the table below. The amounts provided in the table are per well. To cover the attached mRNA in the wells, you may perform RT reaction using up to 80 µl reaction volume by scaling the volume of reagents proportionately.

Reagent	Amount
50 ng/µl Random hexamer	1 µl
10 mM dNTP mix	1 µl
DEPC-treated water	8 µl
10X RT Buffer	2 µl
25 mM $MgCl_2$	4 µl
0.1 mM DTT	2 µl
RNaseOUT™ (40 U/µl)	1 µl
SuperScript™ III RT enzyme (200 U/µl)	1 µl
Total volume	20 µl

2. Remove the Wash Buffer (Step 5 of **Washing mRNA**, previous page) and immediately add 20 µl of the RT-Solution Mix prepared as above to each well of the plate to perform 20 µl (or up to 80 µl) RT reactions.
3. Mix well and cover the mRNA Catcher *PLUS* ™ plate with adhesive aluminum foil plate cover (see page 1 for catalog no).
4. Perform the RT reaction by incubating the plate in a thermocycler suitable for 96-well plates and equipped with heated lids as follows: at 25°C for 10 minutes, at 50°C for 50 minutes, at 85°C for 5 minutes. Transfer the plate on ice.

   The synthesized cDNA in solution is ready for PCR or can be stored at -20°C.

   Note: For blood samples, after cDNA synthesis reaction, transfer the entire cDNA solution into a clean 96-well plate.

For PCR: Use 1-5 µl of the synthesized cDNA sample to perform PCR in a 25-50 µl reaction volume.

For qPCR: Dilute the cDNA from the RT reaction with the same amount of RNase/DNase-free water and mix well. Use 5-10 µl of the diluted cDNA to perform qRT-PCR.

The mRNA Catcher™ *PLUS* system is suitable for cDNA synthesis using a PCR cycler. The mRNA Catcher™ *PLUS* polypropylene plate is compatible with PCR cyclers from all major suppliers. For the subsequent qPCR reaction, use optical tubes or plates, suitable for the PCR cycler of your choice.

Troubleshooting

Problem	Cause	Solution
Low mRNA yield	Incomplete lysis of cells or tissue	Use the appropriate lysis protocol based on the starting material. You may need to use more starting material.
	LNA™ was not covered by the sample	Use up to 80 µl of sample volume to cover the LNA™ for optimal binding efficiency of mRNA in your sample
mRNA degraded	RNA contaminated with RNase	Follow the guidelines on page 2 to prevent RNase contamination.
Low PCR product yield when cDNA synthesis was performed in the plate	The purified mRNA dried out because the RT-Solution Mix was not added immediately after removing the Wash Buffer (W15).	Prepare the RT-Solution Mix before removing the Wash Buffer (W15) in Step 5, Washing Step. Add the RT-Solution Mix IMMEDIATELY after removing the Wash Buffer (W15).
Bloodstain on wells after purification from blood samples	Amount of Wash Buffer (W15) during Washing Step did not cover bloodstain on the well walls	Increase the volume Wash Buffer (W15) to cover the bloodstain in wells (Step 2, Washing Step). Doing this, the amount of Wash Buffer (W15) provided in the kit may not be enough to perform 96 purifications.