Third Edition **December 2014**

TurboCapture™ mRNA Handbook

TurboCapture 96 mRNA Kit

TurboCapture 384 mRNA Kit

For rapid and easy mRNA purification from cultured cells in high-throughput applications

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QIAGEN Sample and Assay Technologies

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Kit Contents

TurboCapture 96 mRNA Kit	**(1)**	**(5)**
Catalog no.	**72250**	**72251**
Number of preps	**1 x 96**	**5 x 96**
TurboCapture 96 mRNA Plate (with lid)	1	5
AlumaSeal™ II Sealing Film	3	15
Buffer TCL (contains RNase inhibitors)	10 ml	125 ml
Buffer TCW	34 ml	2 x 250 ml
Buffer TCE	10 ml	125 ml
Handbook	1	1

TurboCapture 384 mRNA Kit	**(5)**
Catalog no.	**72271**
Number of preps	**5 x 384**
TurboCapture 384 mRNA Plate (with lid)*	5
Buffer TCL (contains RNase inhibitors)	125 ml
Buffer TCW	2 x 250 ml
Buffer TCE	125 ml
Handbook	1

* We recommend sealing the plate with AlumaSeal II sealing film during mRNA elution and cDNA synthesis. AlumaSeal II sealing film is not included in the kit, but can be ordered separately from Excel Scientific (www.excelscientific.com; cat. no. AFS-25) or Sigma-Aldrich (www.sigmaaldrich.com; cat. no. Z707422).

Storage

Store TurboCapture mRNA Kits at 2–8°C. **Do not freeze.** When stored under these conditions, performance is guaranteed for at least 12 months. Keep TurboCapture plates sealed until required, since excessive exposure to air may affect their performance. TurboCapture buffers can be stored at room temperature (15–25°C) if desired.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of TurboCapture mRNA Kits is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

TurboCapture mRNA Kits are intended for molecular biology applications. These products are neither intended for the diagnosis, prevention, or treatment of a disease, nor have they been validated for such use either alone or in combination with other products. Therefore, the performance characteristics of the products for clinical use (i.e., diagnostic, prognostic, therapeutic, or blood banking) are unknown.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN® product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding TurboCapture mRNA Kits or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact

us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

CAUTION: DO NOT add bleach or acidic solutions directly to waste containing Buffer TCL.

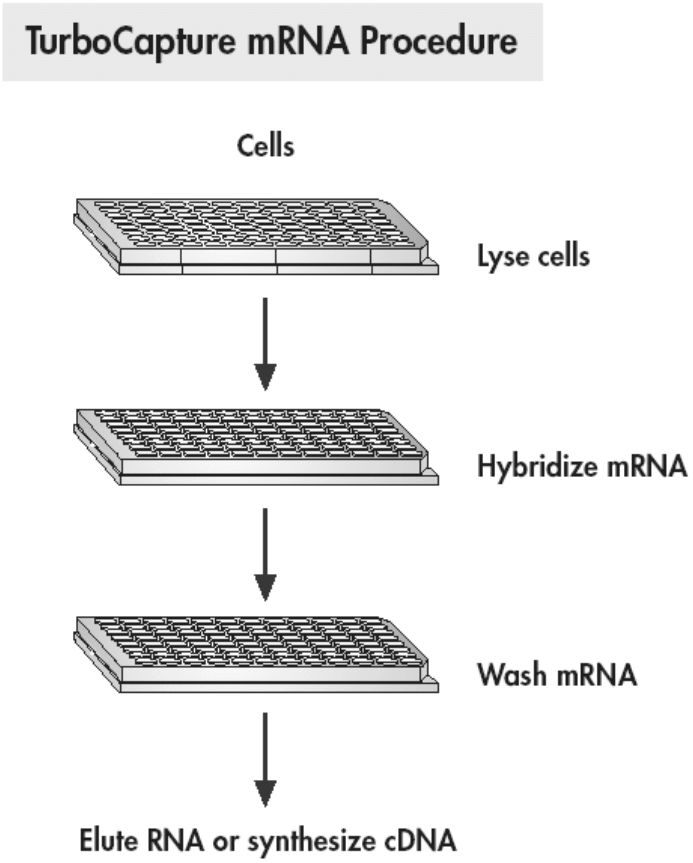
Buffer TCL contains guanidine thiocyanate, which can form highly reactive compounds when combined with bleach. If liquid containing this buffer is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Introduction

TurboCapture mRNA Kits provide rapid and easy purification of mRNA from cultured cells, including suspension and adherent cells. mRNA can also be purified from total RNA. The kits allow parallel processing of large numbers of samples and can be automated, making them ideally suited for high-throughput applications, such as target validation by siRNA screening. In addition, the kits streamline the gene expression analysis workflow by making it possible to carry out mRNA purification, cDNA synthesis, and PCR or real-time PCR in the same reaction vessel.

Principle and procedure

Rapid, cost-effective mRNA purification with minimal hands-on time is achieved using unique oligonucleotide immobilization technology (see flowchart). Cell lysates are added to the wells of a TurboCapture 96 plate (96 wells) or TurboCapture 384 plate (384 wells), and mRNA is allowed to hybridize to the immobilized oligo-dT in each well. Contaminants are washed away, and the isolated mRNA is then either used directly in cDNA synthesis or eluted and quantified.



TurboCapture plates are compatible with most thermal cyclers and most real-time PCR cyclers (e.g., instruments from Applied Biosystems). This allows mRNA hybridization and subsequent cDNA synthesis and PCR or real-time PCR to take place in the same plate, minimizing sample handling and reducing consumable costs. cDNA can be synthesized while mRNA remains hybridized to the well (Figure 1). The immobilized oligo-dT in the well can be used as primer, yielding cDNA which is covalently linked to the well and can be reused several times. Alternatively, soluble oligo-dT primers or random primers can be used to prepare soluble cDNA. For guidelines on synthesizing immobilized cDNA and soluble cDNA, see Appendix C (page 22) and Appendix D (page 25), respectively.

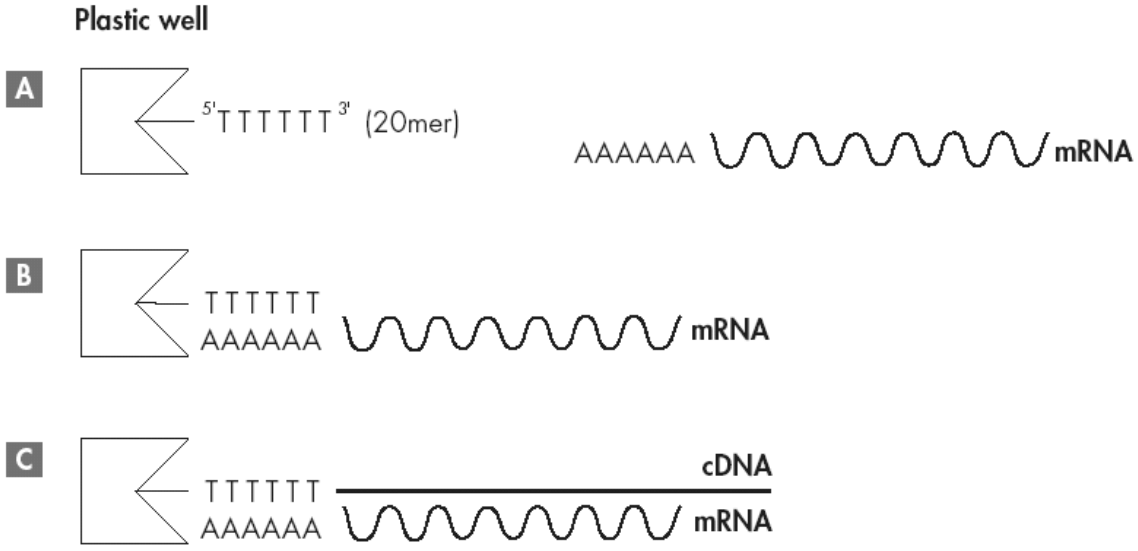


Figure 1. mRNA purification and cDNA synthesis in the same well. **A** Lysate containing poly A^+ mRNA is added to a well containing immobilized oligo-dT. **B** Poly A^+ mRNA hybridizes to the immobilized oligo-dT. **C** While the mRNA is hybridized, cDNA is synthesized using the immobilized oligo-dT as primer. The resulting cDNA is covalently linked to the well. (Alternatively, soluble primers can be used to synthesize soluble cDNA; not shown in figure.)

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

For all protocols

- 14.3 M β-mercaptoethanol (β-ME) (commercially available solutions are usually 14.3 M)
- Sterile, RNase-free tubes and pipet tips
- Phosphate-buffered saline (PBS), pH 7.4
- Water bath, thermal cycler, or incubator
- Adhesive sealing tape to cover TurboCapture plates during storage and PCR
- Optional: Additional sterile AlumaSeal II sealing film to cover TurboCapture plates during incubation at high temperatures (e.g., mRNA elution at 65°C); available from Excel Scientific (www.excelscientific.com; cat. no. AFS-25) or Sigma-Aldrich (www.sigmaaldrich.com; cat. no. Z707422)
- Centrifuge compatible with microplates (may be required to spin down condensation)
- Reagents for reverse transcription and PCR or real-time PCR (we recommend using QIAGEN kits; for details, visit www.qiagen.com/PCR)
- RNase-free 96- or 384-well microplates (for storage of eluted mRNA)

For mRNA purification from suspension cells

- 2x Buffer TCL (see page 33 for ordering information)

For cDNA synthesis

- Sensiscript® RT Kit; when processing larger cell numbers, the QuantiTect® Reverse Transcription Kit can be used instead (see page 34 for ordering information)
- RNase inhibitor
- For synthesis of immobilized cDNA: 10 mM Tris·Cl, pH 7.5
- For synthesis of soluble cDNA: Random primers or oligo-dT primers

For automated sample processing

- TurboCapture Microplate Holder (see Appendix E, page 29, for details, and page 33 for ordering information)

Important Notes

General guidelines

- If using TurboCapture mRNA Kits for the first time, we strongly recommend reading through the handbook before starting. If working with RNA for the first time, read Appendix A (page 19) carefully.
- We recommend using the same liquid volume for mRNA hybridization* and mRNA elution,† and the same reaction volume for cDNA synthesis and PCR if performing these steps directly in the TurboCapture plate. This will ensure that all hybridized mRNA is eluted and that all synthesized cDNA is used in PCR. In addition, we recommend using the lowest liquid volume possible (i.e., the most concentrated sample possible) to ensure the most efficient mRNA hybridization and elution. However, if purifying mRNA from cells, use the lowest possible liquid volume that does not result in highly viscous cell lysate. We recommend starting with a liquid volume of 80 µl (TurboCapture 96 plates) or 20 µl (TurboCapture 384 plates), and reducing this volume where possible. The maximum liquid volume is 80 µl (TurboCapture 96 plates) or 30 µl (TurboCapture 384 plates).
- Allow TurboCapture plates and buffers to reach room temperature (15–25°C) before starting.
- During the procedure, work quickly. Do not leave TurboCapture plates on the benchtop for long periods between steps.
- Counting cells is the most accurate way to quantitate the amount of starting material. As a guide, the number of HeLa cells obtained in various culture vessels after confluent growth is given in Table 1.

* mRNA hybridization corresponds to steps 6 and 7 of the adherent cell protocol (page 11); steps 4 and 5 of the suspension cell protocol (page 13); and steps 3 and 4 of the total RNA protocol (page 15).

† mRNA elution corresponds to steps 11 and 12 of the adherent cell protocol (page 11); steps 9 and 10 of the suspension cell protocol (page 13); and steps 8 and 9 of the total RNA protocol (page 15).

Table 1. Growth area and number of HeLa cells in various culture vessels

Multiwell-plate	Growth area (cm^2)*	Number of cells†
384-well	~0.06	~5 x 10^3
96-well	0.32–0.6	4–5 x 10^4
48-well	1	1 x 10^5
24-well	2	2.5 x 10^5
12-well	4	5 x 10^5

* Per well; varies slightly depending on the supplier.

† Cell numbers are given for HeLa cells (approximate length = 15 μm), assuming confluent growth. Cell numbers will vary for different kinds of animal cells, which vary in length from 10 to 30 μm.

Protocol: Purification of mRNA from Adherent Cells

Important points before starting

- In the procedure below, ▲ refers to instructions for the TurboCapture 96 mRNA Kit, and ● refers to instructions for the TurboCapture 384 mRNA Kit.
- Reduced volumes may be used in steps 5, 6, and 11, depending on cell numbers and cell type (see "General guidelines", page 10).

Procedure

1. **Culture cells overnight in a ▲ 24-, 48-, or 96-well plate or ● 384-well plate.**
2. **Prepare Buffer TCL by adding β-mercaptoethanol to a final concentration of 1% (v/v).**

   For example, add 10 µl β-mercaptoethanol to 990 µl Buffer TCL.

   Note: The buffer should be prepared fresh for each new procedure.
3. **Remove the culture medium from each well.**
4. **Wash the cells using ▲ 100 µl or ● 30 µl PBS per well.**

   Note: Be sure to remove all residual liquid.
5. **Add ▲ 85 µl or ● 25 µl Buffer TCL to each well. Incubate at room temperature (15–25°C) for 5 min.**

   No shaking or mixing is necessary.
6. **Transfer ▲ 80 µl or ● 20 µl of lysate from each well to a TurboCapture plate. Cover the plate with AlumaSeal II sealing film.**

   Avoid creating foam or bubbles.
7. **Incubate the TurboCapture plate at room temperature (15–25°C) for 30–90 min.**

   Incubation for 90 min on an orbital shaker (100 rpm) will usually result in maximum mRNA yields.
8. **Remove the lysates from the TurboCapture plate.**
9. **Wash the TurboCapture plate 3 times using ▲ 100 µl or ● 30 µl Buffer TCW per well.**

   Note: After the third wash, be sure to remove all residual liquid. Then proceed immediately to step 10.
10. **If performing cDNA synthesis in the TurboCapture plate, follow the guidelines in Appendix C (synthesis of immobilized cDNA) or Appendix D (synthesis of soluble cDNA) on pages 22 and 25, respectively. Otherwise, follow the remaining steps to elute mRNA.**

11. **Add ▲ 80 µl or ● 20 µl Buffer TCE to each well of the TurboCapture plate.**
12. **Cover the TurboCapture plate with a fresh sheet of AlumaSeal II sealing film. Incubate at 65°C for 5 min.**

    Incubation in a thermal cycler with a heated lid avoids condensation.
13. **Allow the TurboCapture plate to cool to room temperature for 5 min.**
14. **If necessary, briefly centrifuge the TurboCapture plate to collect condensation in the bottom of the wells.**
15. **Transfer the mRNA eluates to an RNase-free microplate.**
16. **If desired, concentrate the mRNA by ethanol precipitation and/or store at –70°C.**

Protocol: Purification of mRNA from Suspension Cells

Important points before starting

- In the procedure below, ▲ refers to instructions for the TurboCapture 96 mRNA Kit, and ● refers to instructions for the TurboCapture 384 mRNA Kit.
- Reduced volumes may be used in steps 4 and 9, depending on cell numbers and cell type (see "General guidelines", page 10).

Procedure

1. **Culture cells overnight in a ▲ 24-, 48-, or 96-well plate or ● 384-well plate.**
2. **Prepare 2x Buffer TCL (not supplied) by adding β-mercaptoethanol to a final concentration of 2% (v/v).**

   For example, add 20 µl β-mercaptoethanol to 980 µl of 2x Buffer TCL.

   Note: The buffer should be prepared fresh for each new procedure.
3. **To each well, add 1 volume of 2x Buffer TCL to 1 volume of culture medium. Mix by pipetting up and down.**

   The final concentration of Buffer TCL is 1x.
4. **Transfer ▲ 80 µl or ● 20 µl of lysate from each well to a TurboCapture plate. Cover the plate with AlumaSeal II sealing film.**

   Avoid creating foam or bubbles.
5. **Incubate the TurboCapture plate at room temperature (15–25°C) for 30–90 min.**

   Incubation for 90 min on an orbital shaker (100 rpm) will usually result in maximum mRNA yields.
6. **Remove the lysates from the TurboCapture plate.**
7. **Wash the TurboCapture plate 3 times using ▲ 100 µl or ● 30 µl Buffer TCW per well.**

   Note: After the third wash, be sure to remove all residual liquid. Then proceed immediately to step 8.
8. **If performing cDNA synthesis in the TurboCapture plate, follow the guidelines in Appendix C (synthesis of immobilized cDNA) or Appendix D (synthesis of soluble cDNA) on pages 22 and 25, respectively. Otherwise, follow the remaining steps to elute mRNA.**
9. **Add ▲ 80 µl or ● 20 µl Buffer TCE to each well of the TurboCapture plate.**

10. **Cover the TurboCapture plate with a fresh sheet of AlumaSeal II sealing film. Incubate at 65°C for 5 min.**

    Incubation in a thermal cycler with a heated lid avoids condensation.

11. **Allow the TurboCapture plate to cool to room temperature (15–25°C) for 5 min.**
12. **If necessary, briefly centrifuge the TurboCapture plate to collect condensation in the bottom of the wells.**
13. **Transfer the mRNA eluates to an RNase-free microplate.**
14. **If desired, concentrate the mRNA by ethanol precipitation and/or store at –70°C.**

Protocol: Purification of mRNA from Total RNA

Important points before starting

- The quality of the mRNA that is purified strongly depends on the quality of the starting total RNA sample.
- In the procedure below, ▲ refers to instructions for the TurboCapture 96 mRNA Kit, and ● refers to instructions for the TurboCapture 384 mRNA Kit.
- Reduced volumes may be used in steps 3 and 8, depending on RNA amount (see "General guidelines", page 10).

Procedure

1. **Prepare Buffer TCL by adding β-mercaptoethanol to a final concentration of 1% (v/v).**

   For example, add 10 µl β-mercaptoethanol to 990 µl Buffer TCL.

   Note: The buffer should be prepared fresh for each new procedure.

2. **Dilute each total RNA sample with 9 volumes of Buffer TCL, and mix by pipetting up and down.**

   For example, add 8.5 µl total RNA to 76.5 µl Buffer TCL, or add 2.5 µl total RNA to 22.5 µl Buffer TCL.

3. **Transfer ▲ 80 µl or ● 20 µl of each sample to a TurboCapture plate. Cover the plate with AlumaSeal II sealing film.**

   Avoid creating foam or bubbles.

   If the volume of diluted total RNA is less than ▲ 80 µl or ● 20 µl, increase the volume by adding more Buffer TCL.

4. **Incubate the TurboCapture plate at room temperature (15–25°C) for 30–90 min.**

   Incubation for 90 min on an orbital shaker (100 rpm) will usually result in maximum mRNA yields.

5. **Remove the liquid from the TurboCapture plate.**
6. **Wash the TurboCapture plate 3 times using ▲ 100 µl or ● 30 µl Buffer TCW per well.**

   Note: After the third wash, be sure to remove all residual liquid. Then proceed immediately to step 7.

7. **If performing cDNA synthesis in the TurboCapture plate, follow the guidelines in Appendix C (synthesis of immobilized cDNA) or Appendix D (synthesis of soluble cDNA) on pages 22 and 25, respectively. Otherwise, follow the remaining steps to elute mRNA.**

8. **Add ▲ 80 µl or ● 20 µl Buffer TCE to each well of the TurboCapture plate.**
9. **Cover the TurboCapture plate with a fresh sheet of AlumaSeal II sealing film. Incubate at 65°C for 5 min.**

   Incubation in a thermal cycler with a heated lid avoids condensation.
10. **Allow the TurboCapture plate to cool to room temperature for 5 min.**
11. **If necessary, briefly centrifuge the TurboCapture plate to collect condensation in the bottom of the wells.**
12. **Transfer the mRNA eluates to an RNase-free microplate.**
13. **If desired, concentrate the mRNA by ethanol precipitation and/or store at –70°C.**

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
Little or no mRNA eluted	
Not enough starting material	Determine the appropriate amount of starting material by performing an experiment in which mRNA is purified from increasing amounts of starting material.
mRNA degraded	
a) Samples not handled properly	Be sure to work quickly during the mRNA purification procedure: if samples are not processed immediately after lysis, the purified mRNA may be of reduced integrity. During mRNA hybridization, avoid possible RNase contamination by covering the TurboCapture plate with AlumaSeal II sealing film.
b) Buffers contaminated with RNases	Although the buffers supplied with TurboCapture mRNA Kits are guaranteed to be free of RNase, RNases can be introduced during the mRNA purification procedure. Refer to Appendix A (page 19), which gives advice on handling RNA.

Appendix A: General Remarks on Handling RNA

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure. In order to create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and nondisposable vessels and solutions while working with RNA.

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice when aliquots are pipetted for downstream applications.

Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases.

Nondisposable plasticware

Nondisposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH,* 1 mM EDTA* followed by RNase-free water (see "Solutions", page 20). Alternatively, chloroform-resistant plasticware can be rinsed with chloroform* to inactivate RNases.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent,* thoroughly rinsed, and oven baked at 240°C for at least 4 hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate). Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight (12 hours) at 37°C, and then autoclave or heat to 100°C for 15 minutes to eliminate residual DEPC.

Electrophoresis tanks

Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS*), thoroughly rinsed with RNase-free water, and then rinsed with ethanol*† and allowed to dry.

Solutions

Solutions (water and other solutions) should be treated with 0.1% DEPC. DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Add 0.1 ml DEPC to 100 ml of the solution to be treated and shake vigorously to bring the DEPC into solution. Let the solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris* buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO_2. When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

Note: TurboCapture buffers are guaranteed RNase-free without using DEPC treatment and are therefore free of any DEPC contamination.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

† Plastics used for some electrophoresis tanks are not resistant to ethanol. Take proper care and check the supplier's instructions.

Appendix B: Storage, Quantification, and Determination of Quality of mRNA

Storage of mRNA

Purified mRNA may be stored at –70°C in Buffer TCE.

Quantification of RNA

Since poly A^+ mRNA accounts for only 1–5% of total RNA, it may be difficult to determine amounts photometrically. Small amounts of mRNA can be quantified using the QIAxcel system (www.qiagen.com/QIAxcel) or Agilent® 2100 bioanalyzer, quantitative RT-PCR, or fluorometric quantification.

DNA contamination

No currently available purification method can guarantee that RNA is completely free of DNA. While TurboCapture mRNA Kits will remove the vast majority of cellular DNA, trace amounts may still remain, depending on the amount and nature of the sample.

For analysis of very low abundance targets, any interference by residual DNA contamination can be detected by performing real-time RT-PCR control experiments in which no reverse transcriptase is added prior to the PCR step.

To prevent any interference by DNA in real-time RT-PCR applications, such as with Applied Biosystems® and LightCycler® instruments, we recommend designing primers that anneal at intron splice junctions so that genomic DNA will not be amplified. QuantiTect Primer Assays from QIAGEN (www.qiagen.com/GeneGlobe) are designed for SYBR® Green-based real-time RT-PCR analysis of RNA sequences (without detection of genomic DNA) where possible.

Appendix C: Synthesizing Immobilized cDNA Using the Sensiscript RT Kit

The procedure in this appendix describes how to synthesize cDNA that is covalently linked to a TurboCapture plate. Before starting this procedure, purify mRNA from your samples according to one of the following protocols:

- Follow steps 1–9 of the adherent cell protocol (page 12)
- Follow steps 1–7 of the suspension cell protocol (page 14)
- Follow steps 1–6 of the total RNA protocol (page 16)

Important points before starting

- We recommend using the Sensiscript RT Kit to synthesize cDNA. For larger cell numbers (i.e., cells grown in 12- or 24-well plates), the QuantiTect Reverse Transcription Kit, which provides integrated genomic DNA removal, can also be used.
- The reaction mix for reverse transcription contains reverse transcriptase, nucleotides, and buffer, **but no primers**. The immobilized oligo-dT in the TurboCapture plate serves as the primer.
- RNase inhibitor is a 50 kDa protein that strongly inhibits RNases A, B, and C, as well as human placental RNases. For best results, we highly recommend using RNase inhibitor to minimize the risk of RNA degradation during experimental setup. RNase inhibitor is commonly supplied at a concentration of 40 units/µl.
- For optimal results, the volume of the reverse-transcription reaction mix should be the same as that of the mRNA hybridization reaction.* If a smaller volume of reaction mix is used, not all the bound mRNA will be available as template for cDNA synthesis.

Procedure

C1. Prepare reverse-transcription reaction mix (without any primers) as described in Table 2. After removing all remaining Buffer TCW from the TurboCapture plate, immediately add the reaction mix to each well.

* mRNA hybridization corresponds to steps 6 and 7 of the adherent cell protocol (page 11); steps 4 and 5 of the suspension cell protocol (page 13); and steps 3 and 4 of the total RNA protocol (page 15).

Table 2. Preparing reaction mix using the Sensiscript RT Kit for synthesis of immobilized cDNA

Component	Volume/reaction for TurboCapture 384 plate	Volume/reaction for TurboCapture 96 plate	Final concentration
10x Buffer RT	2 µl	8 µl	1x
dNTP Mix* (5 mM each dNTP)	2 µl	8 µl	0.5 mM each dNTP
RNase inhibitor (10 units/µl)	1 µl	4 µl	0.5 unit/µl
Sensiscript Reverse Transcriptase	1 µl	1 µl or 4 µl†	–
RNase-free water	14 µl	59 µl or 56 µl†	–
Total volume	**20 µl**	**80 µl**	**–**

* Contains dATP, dCTP, dGTP, and dTTP.

† If mRNA was purified from up to about 1 x 10^5 cells (i.e., cells grown in a 48- or 96-well plate), use 1 µl Sensiscript Reverse Transcriptase and 59 µl water. If mRNA was purified from higher cell numbers, use 4 µl Sensiscript Reverse Transcriptase and 56 µl water.

C2. Cover the TurboCapture plate with AlumaSeal II sealing film. Incubate at 37°C for 60 min.

C2. Remove the reverse-transcription reaction mix.

The synthesized cDNA is covalently linked to the TurboCapture plate.

C3. Wash the TurboCapture plate 3 times using 10 mM Tris·Cl, pH 7.5 (100 µl per well of a TurboCapture 96 plate, or 30 µl per well of a TurboCapture 384 plate).

C4. After removing all remaining liquid from the TurboCapture plate, immediately add PCR mix to each well.

For optimal results, the volume of the PCR mix should be the same as that of the reverse-transcription reaction.

To prepare the PCR mix, follow the instructions supplied with the PCR kit you are using (to order PCR kits from QIAGEN, visit www.qiagen.com/PCR). The PCR mix contains DNA polymerase, nucleotides, buffer, and primers for the target gene.

C5. Cover the TurboCapture plate with adhesive sealing tape (not supplied). Perform PCR according to the instructions supplied with the PCR kit.

TurboCapture 96 plates are compatible with most 96-well thermal cyclers and are optically suited for real-time cyclers. TurboCapture 384 plates are compatible with most 384-well thermal cyclers and are optically suited for real-time cyclers.

Note: The TurboCapture plate containing immobilized cDNA can be reused. Be sure to wash the wells 3 times with 10 mM Tris·Cl, pH 7.5 between PCRs (100 µl per well of a TurboCapture 96 plate; 30 µl per well of a TurboCapture 384 plate). The heating steps during PCR may result in cDNA strand breaks, which will limit reuse of the immobilized cDNA, especially for amplicons distal to the 3' end of mRNA transcripts. No more than 3 rounds of PCR can be performed, since the plate may crack.

Appendix D: Synthesizing Soluble cDNA Using the Sensiscript RT Kit

This appendix contains guidelines on how to synthesize soluble cDNA in TurboCapture plates. Guidelines and examples are provided for:

- **One-step cDNA synthesis**: In this method, most of the mRNA remains bound to the wells of the TurboCapture plate. Therefore, reverse transcription is performed using random primers instead of oligo-dT primers. Sequences directly adjacent to the 3' end of mRNA transcripts may be underrepresented in the soluble cDNA. This method provides faster results, as an incubation step to release bound mRNA is not required.
- **Two-step cDNA synthesis**: In this method, bound mRNA is released from the wells of the TurboCapture plate prior to cDNA synthesis. Reverse transcription is performed using gene-specific primers, random primers, oligo-dT primers, or a mix of random and oligo-dT primers. This method enables better representation of sequences close to the 3' end of mRNA transcripts.

Before starting cDNA synthesis, purify mRNA from your samples according to one of the following protocols:

- Follow steps 1–9 of the adherent cell protocol (page 12)
- Follow steps 1–7 of the suspension cell protocol (page 14)
- Follow steps 1–6 of the total RNA protocol (page 16)

Important points before starting

- We recommend using the Sensiscript RT Kit to synthesize cDNA. For larger cell numbers (i.e., cells grown in 12- or 24-well plates), the QuantiTect Reverse Transcription Kit, which provides integrated genomic DNA removal, can also be used.
- For optimal cDNA synthesis, use oligo-dT primers and gene-specific primers at a final concentration of 0.1–1 µM each, and random nonamers at a final concentration of 10 µM.
- RNase inhibitor is a 50 kDa protein that strongly inhibits RNases A, B, and C, as well as human placental RNases. For best results, we highly recommend using RNase inhibitor to minimize the risk of RNA degradation during experimental setup. RNase inhibitor is commonly supplied at a concentration of 40 units/µl.

- For optimal results, the volume of the reverse-transcription reaction mix should be the same as that of the mRNA hybridization reaction.* If a smaller volume of reaction mix is used, not all the bound mRNA will be available as template for cDNA synthesis.

One-step cDNA synthesis using random primers

- Prepare reverse-transcription reaction mix according to Table 3.

Table 3. Preparing reaction mix using the Sensiscript RT Kit for one-step cDNA synthesis

Component	Volume/reaction for TurboCapture 384 plate	Volume/reaction for TurboCapture 96 plate	Final concentration
10x Buffer RT	2 µl	8 µl	1x
dNTP Mix* (5 mM each dNTP)	2 µl	8 µl	0.5 mM each dNTP
Random primer (100 µM)	2 µl	8 µl	10 µM
RNase inhibitor (10 units/µl)	1 µl	4 µl	0.5 unit/µl
Sensiscript Reverse Transcriptase	1 µl	1 µl or 4 µl†	–
RNase-free water	12 µl	51 µl or 48 µl†	–
Total volume	**20 µl**	**80 µl**	**–**

* Contains dATP, dCTP, dGTP, and dTTP.

† If mRNA was purified from up to about 1 x 10^5 cells (i.e., cells grown in a 48- or 96-well plate), use 1 µl Sensiscript Reverse Transcriptase and 51 µl water. If mRNA was purified from higher cell numbers, use 4 µl Sensiscript Reverse Transcriptase and 48 µl water.

- After removing all remaining Buffer TCW from the TurboCapture plate, immediately add reverse-transcription reaction mix to each well (80 µl for a TurboCapture 96 plate; 20 µl for a TurboCapture 384 plate).
- Cover the TurboCapture plate with AlumaSeal II sealing film. Incubate at 37°C for 60 min.

* mRNA hybridization corresponds to steps 6 and 7 of the adherent cell protocol (page 11); steps 4 and 5 of the suspension cell protocol (page 13); and steps 3 and 4 of the total RNA protocol (page 15).

- Store the reverse-transcription reactions on ice and proceed directly with PCR, or for long-term storage, store the reverse-transcription reactions at –15 to –30°C.

Two-step cDNA synthesis using oligo-dT primers

- Prepare incubation mix and reverse-transcription mix according to Tables 4 and 5.

Table 4. Preparing incubation mix for two-step cDNA synthesis

Component	Volume/reaction for TurboCapture 96 plate*	Final concentration
Buffer TCE	60 µl	–
dNTP Mix† (5 mM each dNTP)	8 µl	0.5 mM each dNTP
Oligo-dT primer (10 µM)	2 µl	0.25 µM
Total volume	**70 µl**	**–**

* This amount of incubation mix is suitable for mRNA purified from up to about 1 x 10^5 cells (i.e., cells grown in a 48- or 96-well plate).

† Contains dATP, dCTP, dGTP, and dTTP.

Table 5. Preparing reverse-transcription mix using the Sensiscript RT Kit for two-step cDNA synthesis

Component	Volume/reaction for TurboCapture 96 plate	Final concentration
10x Buffer RT	8 µl	1x
RNase inhibitor (10 units/µl)	1 µl	0.125 unit/µl
Sensiscript Reverse Transcriptase	1 µl	–
Total volume	**10 µl**	**–**

- After removing all remaining Buffer TCW from the TurboCapture plate, immediately add 70 µl incubation mix to each well.
- Cover the TurboCapture plate with AlumaSeal II sealing film. Incubate at 65°C for 5 min to elute mRNA from the wells of the plate.

- Cool the TurboCapture plate to room temperature (15–25°C), and add 10 µl reverse-transcription mix to each well.
- Cover the TurboCapture plate with AlumaSeal II sealing film. Incubate at 37°C for 60 min.
- Store the reverse-transcription reactions on ice and proceed directly with PCR, or for long-term storage, store the reverse-transcription reactions at –15 to –30°C.

Appendix E: Guidelines on Using TurboCapture Microplate Holders

Reliable liquid handling on robotic workstations is assured when TurboCapture microplate holders are used to support TurboCapture plates. A TurboCapture microplate holder is a spring-loaded platform that allows a TurboCapture plate to move downwards when a moving pipet tip touches the bottom of a well. Aspiration and dispensing can be performed at the bottom of a well without the pipet tip sealing to the well, enabling:

- Dispensing of low volumes of liquid into the well without bubble formation
- Complete aspiration of liquid from the well without tip crashes

There are 2 types of TurboCapture microplate holder: type A, which is compatible with many robotic workstations, and type B, which is for use with the Biomek® 2000. When using a TurboCapture microplate holder with TurboCapture 96 plates, an appropriate support base is required; for further information, contact QIAGEN Technical Services. This appendix provides guidelines on installing and using these microplate handlers.

TurboCapture Microplate Holder, Type A

This microplate holder is compatible with a wide range of robotic workstations, since its dimensions are based on SBS (Society for Biomolecular Screening) specifications.

Table 6 provides the exterior dimensions of the microplate holder. Programming your robotic workstation using these dimensions will allow pipet tips to move safely around the microplate holder.

Table 6. Exterior dimensions of TurboCapture Microplate Holder, Type A

Base:	
■ Length	127.87 mm
■ Width	85.58 mm
Top:	
■ Length	136.33 mm
■ Width	93.84 mm
Overall uncompressed height (including tabs)	41.50 mm

Table 7 shows the different positions in the Z-axis of a microplate placed on top of the microplate holder. When programming your robotic workstation to pipet at the bottom of the microplate well, we recommend entering the optimal Z-axis displacement setting of 29.60 mm. With this setting, the springs of the

microplate holder are half-compressed, providing 1.65 mm of flexibility in either direction of the Z-axis and resolving most problems associated with warped microplates and/or uneven pipet tips.

Table 7. Z-axis positions of a microplate on the TurboCapture Microplate Holder, Type A

Z-axis displacement of microplate (springs uncompressed)	31.25 mm
Z-axis displacement of microplate (springs fully compressed):	27.95 mm
Optimal Z-axis displacement setting for microplate (springs half-compressed):	29.60 mm

The following guidelines should be followed when using the microplate holder:

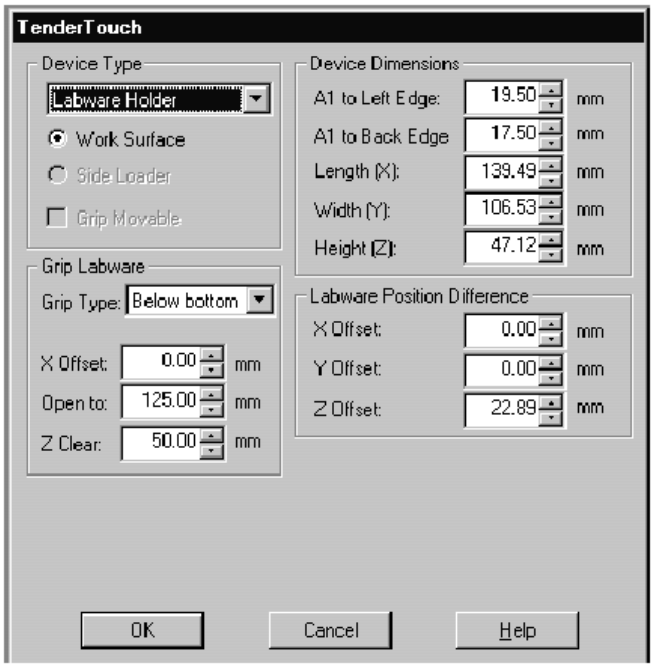
- For optimal results, use slower speeds for dispensing and aspiration of liquid.
- If the microplate contains unused wells, the microplate may not have an even distribution of weight. Therefore, the microplate may not be level when placed on the microplate holder. This is usually not a problem, as the microplate holder is designed to overcome this issue.
- We do not recommend using the microplate holder with deep-well blocks.
- When aspirating larger volumes of liquid, some residual liquid may remain on the walls of the well. This issue may be resolved by using a microplate made of a different material or by reducing the aspiration speed.
- When programming your robotic workstation, ensure that the volume of liquid aspirated is greater than the volume of liquid dispensed. This avoids the creation of bubbles.

TurboCapture Microplate Holder, Type B

The dimensions of the microplate holder are programmed into the Biomek 2000 as follows:

- In the Bioworks edit module, select “Devices” in the “Edit” menu.
- In the “Edit Device” dialog box which appears, highlight “Labware Holder” and click the “Copy” button.

- In the “Device Copy” dialog box which appears, enter the following information and click the “OK” button:
  - New Name: *TurboCapture*
  - Author: *QIAGEN*
  - Keyword: *Worksurface*
- Highlight “TurboCapture” in the “Edit Device” dialog box and click the “Edit” button.
- In the “TurboCapture” dialog box which appears, enter the following information and click the “OK” button:



  This information will have to be imported into any Lab Book requiring its use.

The microplate holder is loaded onto the Biomek 2000 as follows:

- Orient the microplate holder so that the side labeled “FRONT” faces the front of the Biomek 2000 work surface.
- Place the microplate holder into the desired deck location by inserting the detent pins of the microplate holder into the orientation holes of the deck.
- Place a standard Biomek 2000 (or ORCA® accessible) labware holder on top of the microplate holder by inserting the detent pins of the labware holder into the orientation holes of the microplate holder.

The following guidelines should be followed when using the microplate holder:

- When writing a method, add the microplate holder to the desired deck location in the initial configuration. In the method, labware can be placed directly on top of the microplate holder; no labware holders are necessary (the microplate holder itself represents a modified labware holder).
- When writing a method to pipet at the bottom of the microplate well, we recommend using a setting in which the springs of the microplate holder are half-compressed. This provides 1.5 mm of flexibility in either direction of the Z-axis.
- For optimal results, set the speed to 6 or below for dispensing and aspiration of liquid.
- Depending on the labware holder placed on top of the microplate holder and depending on the distribution of liquid in the microplate, the microplate may not be level. This is usually not a problem, as the microplate holder is designed to overcome this issue.
- We do not recommend using the microplate holder with deep-well blocks.
- When aspirating larger volumes of liquid, some residual liquid may remain on the walls of the well. This issue may be resolved by using a microplate made of a different material or by reducing the aspiration speed to below 6.
- If handling low volumes of liquid, ensure that in the “Pipette Transfer” dialog box and under “Dispense Type”, “To Deliver” is selected. If “To Contain” is selected, air bubbles will be introduced into the sample.

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Ordering Information

Product	Contents	Cat. no.
TurboCapture 96 mRNA Kit (1)	1 x TurboCapture 96 mRNA Plate, and RNase-Free Buffers	72250
TurboCapture 96 mRNA Kit (5)	5 x TurboCapture 96 mRNA Plates, and RNase-Free Buffers	72251
TurboCapture 384 mRNA Kit (5)	5 x TurboCapture 384 mRNA Plates, and RNase-Free Buffers	72271
Accessories		
Buffer TCL (10 ml)	10 ml of lysis buffer for TurboCapture mRNA Kits	1031573
Buffer TCL (125 ml)	125 ml of lysis buffer for TurboCapture mRNA Kits	1031576
Buffer TCL, 2x (8.5 ml)	8.5 ml of 2x lysis buffer for TurboCapture mRNA Kits	1031586
Buffer TCW (34 ml)	34 ml of wash buffer for TurboCapture mRNA Kits	1031575
Buffer TCW (250 ml)	250 ml of wash buffer for TurboCapture mRNA Kits	1031578
Buffer TCE (125 ml)	125 ml of elution buffer for TurboCapture mRNA Kits	1031577
Oligo-dT Primers (100 µl)	100 µl of oligo-dT primers (0.4 µg/µl) for cDNA synthesis with TurboCapture mRNA Kits	79237
TurboCapture Microplate Holder, Type A	Holder for accommodating TurboCapture plates on robotic workstations	79238
TurboCapture Microplate Holder, Type B	Holder for accommodating TurboCapture plates on the Biomek 2000 robotic workstation	79239

Product	Contents	Cat. no.
Related products		
Sensiscript RT Kit — for reverse transcription using <50 ng RNA per reacton		
Sensiscript RT Kit (50)*	For 50 x 20 µl reactions: Sensiscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix (contains 5 mM each dNTP), and RNase-Free Water	205211
QuantiTect Reverse Transcription Kit — for fast reverse transcription for sensitive real-time two-step RT-PCR		
QuantiTect Reverse Transcription Kit (50)*	For 50 x 20 µl reactions: gDNA Wipeout Buffer, Quantiscript® Reverse Transcriptase, Quantiscript RT Buffer, RT Primer Mix, and RNase-Free Water	205311

The Sensiscript RT Kit and QuantiTect Reverse Transcription Kit are intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Kits that deliver highly specific and sensitive results in end-point PCR and real-time PCR are also available from QIAGEN — to find out more, visit www.qiagen.com/PCR.

* Other kit sizes available; please inquire.

Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the TurboCapture 96 mRNA Kit or TurboCapture 384 mRNA Kit to the following terms:

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