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AllPrep® DNA/mRNA Nano Handbook

For simultaneous purification of genomic DNA and mRNA from low-biomass input samples

Sample to Insight

QIAGEN

Contents

Kit Contents

AllPrep DNA/mRNA Nano **Catalog no.** **No. of reactions**	 **80272** **12**
AdnaTest Lysis/Binding Buffer	3 x 2 ml
Oligo(dT)$_{25}$ Beads	355 µl
RNA Purification Buffer A	4 ml
RNA Purification Buffer B	4 ml
Tris·Cl Buffer	2 ml
Buffer AVE	1.9 ml
Elution Buffer APN	1.5 ml
RNase-Free Water	10 ml
Proteinase K	2 x 250 µl
Binding Buffer APN	1.8 ml
Magnetic Bead Suspension APN	360 µl
Wash Buffer APN1	6 ml
Wash Buffer APN2	12 ml
Collection Tubes (1.5 ml)	3 x 24
Quick-Start Protocol	1

Shipping and Storage

The AllPrep DNA/mRNA Nano Kit is shipped at 2–8°C. It should be stored immediately upon receipt at 2–8°C. Under these conditions, the components are stable for 1 year without showing any reduction in performance and quality.

Intended Use

The AllPrep DNA/mRNA Nano Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

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.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the AllPrep DNA/mRNA Nano Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The AllPrep DNA/mRNA Nano Kit is designed to purify genomic DNA and mRNA simultaneously from the same, precious sample. High quality genomic DNA and mRNA are purified from the entire sample, in contrast to other procedures where either the biological sample or the purified total nucleic acids are divided into two before being processed separately. The kit is compatible with small amounts of low-biomass samples such as circulating tumor cells (CTCs), rare cells, and low number of cells of animal and human origin.

The AllPrep DNA/mRNA Nano Kit allows the parallel processing of multiple samples in less than 75 min. Methods involving the use of toxic substances such as phenol and/or chloroform, or time-consuming and tedious methods such as alcohol precipitation, are replaced by the AllPrep DNA/mRNA procedure.

The mRNA as well as the genomic DNA purification use magnetic bead-based workflows and therefore require magnetic racks (e.g., the AdnaMag-S magnetic rack) to perform the procedure with no need for centrifugation

The genomic DNA purified with the AllPrep DNA/mRNA Nano procedure is high-molecular-weight DNA of up to 100 kb. The purified DNA is suited for any downstream application such as qPCR, digital PCR (dPCR), pyrosequencing, and NGS.

With the AllPrep DNA/mRNA procedure, messenger RNA is isolated using magnetic Oligo-dT Beads. The purified mRNA is of high quality and can be used in any applications, such as cDNA generation, RT-PCR, real-time RT-PCR, and dPCR, including the AdnaTest procedure for molecular characterization of CTCs.

Principle and procedure

The AllPrep DNA/mRNA procedure integrates QIAGEN's CTC AdnaTest technology for selective enrichment of mRNA using Oligo-dT magnetic beads technology with magnetic bead-based DNA isolation. Efficient purification of high-quality DNA and mRNA is guaranteed, without the need for additional RNase and DNase digestions.

Methods involving the use of toxic substances, such as phenol and/or chloroform, or time-consuming and tedious methods such as alcohol precipitation, are replaced by the AllPrep DNA/mRNA procedure (**Figure 1**).

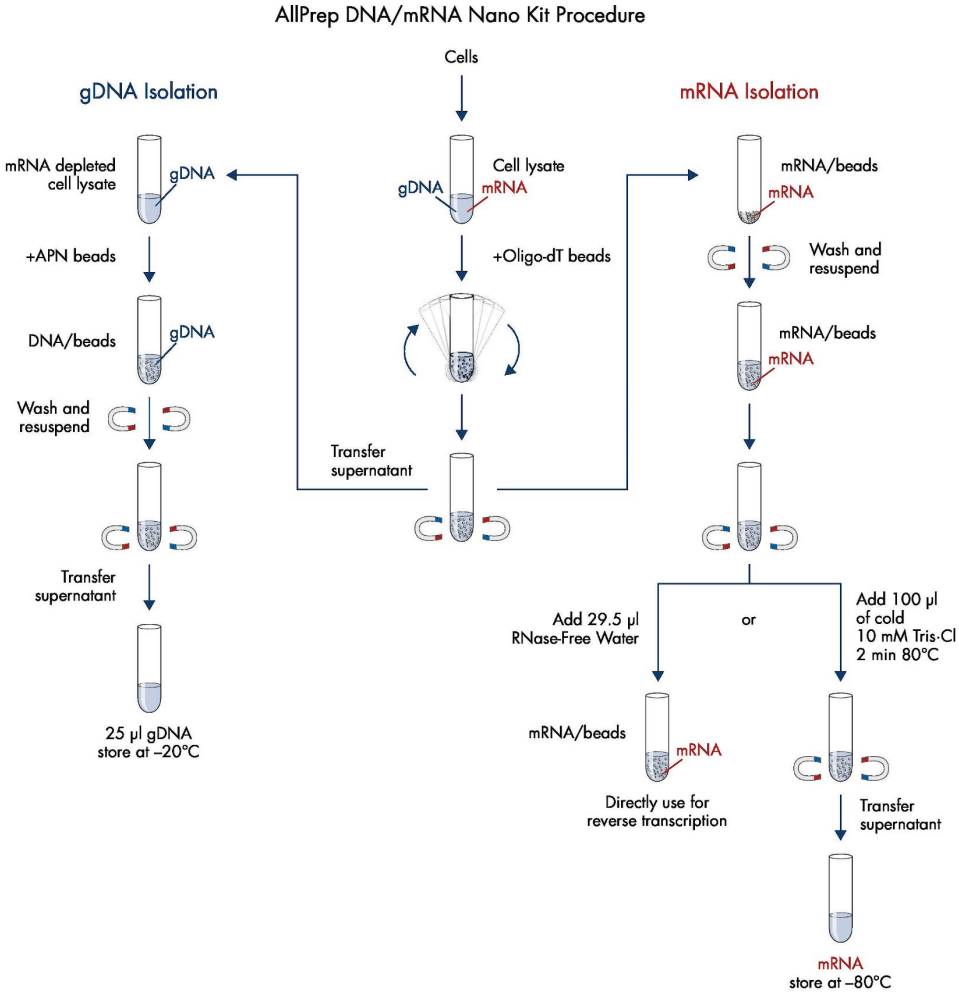

Figure 1. AllPrep DNA/mRNA Nano workflow.

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.

Equipment

- Tube rotator for 1.5 ml tubes (e.g., ELMI Ltd. cat. no. IMIX-03)
- Magnetic particle concentrator AdnaMag-S (cat. no. 399911)
- Thermal block (50°C)
- Centrifuge

Material

- Sterile, RNase-free thin-wall 0.2 ml PCR tubes
- Sterile, RNase-free 1.5 ml reaction tubes (e.g., Sarstedt cat. no. 72.690)
- Pipettes and RNase-free pipette tips with aerosol barrier, suitable for pipetting volumes from 1 to 1000 µl
- Crushed ice
- Optional – if cDNA generation is required
  - SensiScript® RT Kit (cat. no. 205211)
  - Recombinant RNasin®, RNase Inhibitor, 2500 U (Promega cat. no. N2511)

Important Notes

Oligo(dT)$_{25}$ Beads contain sodium azide as preservative. Sodium azide must be removed before using the beads. (See "Things to do before starting:

- Preparation of Oligo(dT)$_{25}$ Beads", page 12).
- The preparation must be performed in the denoted sequence and must comply with all specifications stated in respect of incubation times and incubation temperatures.

Starting material

- Cells must be lysed in 200 µl AdnaTest Lysis/Binding Buffer (provided).
- Use of formalin-fixed samples is not possible.
- Number of cells ranges from a minimum of 1 cell to a maximum of 10,000 cells.

Cultured cells

- After harvesting, cells should be immediately lysed in AdnaTest Lysis Buffer to prevent unwanted changes in the gene expression profile. This highly denaturing lysis buffer inactivates RNases and other proteins to prevent RNA and DNA degradation, as well as downregulation or upregulation of transcripts.

Circulating tumor cells

- CTCs enriched with AdnaTest CTC-Select or other methods need to be lysed in AdnaTest Lysis Buffer immediately. Cell lysates prepared using the AdnaTest CTC-Select Kit can be stored at –30 to –15°C for up to 4 weeks. For CTC isolation using the AdnaTest, follow the procedure described in the AdnaTest CTC-Select handbook (cat. no. 395092).

Rare cells

- Other rare cells may include fetal cells from maternal blood or dissected cells from tissue or fine-needle biopsies. After cells isolation, cells should be lysed immediately using the AdnaTest Lysis Buffer to prevent unwanted changes in the gene expression profile.

Limitations of small samples

- When purifying nucleic acids from particularly small amounts of DNA and RNA, the amount may be too small for quantification by spectrophotometry or even fluorometric assays. In this case, real-time PCR should be used for quantification of mRNA. For the quantification of genomic DNA from human cells, the Investigator® Quantiplex® Pro Kit (cat. no. 387216) is recommended.
- When purifying DNA and RNA from less than 100 cells, stochastic problems with respect to copy number can occur in subsequent NGS. For example, if 20 cells are processed, and DNA is eluted in the recommended minimum volume of 30 µl, there will be less than 1 copy of each genomic DNA allele per microliter. Similarly, some RNA transcripts may be present at very low copy numbers per cell, or only in a fraction of all cells in the sample of interest.

Protocol: Simultaneous Purification of Genomic DNA and mRNA from Low-Biomass Samples

Important points before starting

- If using the AllPrep DNA/mRNA Nano Kit for the first time, read "Important Notes" (page 9).
- Prepare your cells, see section "Starting material" (page 9).
- Oligo(dT)$_{25}$ Beads contain sodium azide as preservative. Sodium azide must be removed before using the beads. (See "Preparation of Oligo(dT)$_{25}$ Beads", page 12).
- Please use the provided 1.5 ml collection tubes only for the protocol step indicated.
- For the optional resuspension of the mRNA/Oligo-dT bead complex (step 12, page 14), please aliquot 400 µl of the provided 10 ml filling of RNase-Free Water for later use in a separate tube.
- Ensure that the AdnaTest Lysis/Binding Buffer is equilibrated to room temperature. If a precipitate is observed, equilibrate the reagent to room temperature and mix until the precipitate is completely dissolved.
- Equilibrate RNA Purification Buffer A and RNA Purification Buffer B to room temperature. Place the Tris·Cl Buffer on ice.
- Adjust a thermal block to 50°C.
- Prepare Elution Buffer AVE/APN. For each sample, mix 22.4 µl Buffer AVE with 7.6 µl Elution Buffer APN. gDNA elution is strongly pH dependent. Make sure that the elution buffer is freshly prepared and does not get longer time contact to air since the buffer pH may increase due to CO_2 content in the air and, therefore, lose its elution ability.
- Make sure not to mix up Elution Buffer APN and Binding Buffer APN.
- Make sure the magnetic beads stay properly in suspension. Intermediate resuspending might be necessary, depending on the number of samples processed.

Things to do before starting

Preparation of Oligo(dT)$_{25}$ Beads

1. Resuspend the Oligo(dT)$_{25}$ Beads thoroughly by pipetting before use.

   Important: Do not vortex.

2. Calculate the volume of the beads required for all samples to be processed (20 µl per sample plus 10% extra volume) and transfer the calculated volume into an RNase-free 1.5 ml reaction tube (not provided).

3. Place the tube into the AdnaMag-S magnetic rack.

   Note: The magnet slider of the AdnaMag-S can be inserted in 2 positions. Always insert the slider with forward-facing white plastic film to make sure that the magnets are next to the reaction tubes.

4. After 1 min, remove the supernatant with a pipette.

5. Wash steps:

   5a. Remove the magnet slider from the AdnaMag-S.
   5b. Add the original volume (step 2) AdnaTest Lysis/Binding Buffer and resuspend the beads by repeated pipetting. Resuspend gently to avoid foaming.
   5c. Insert the magnet slider into the AdnaMag-S.
   5d. After 1 min, remove the supernatant completely.
   5e. Repeat steps 5a to 5d once (two washes in total).

6. Remove the tube from the AdnaMag-S, and resuspend the beads in AdnaTest Lysis/Binding Buffer to the original volume (step 2). Proceed to “mRNA isolation” (page 13).

Procedure

mRNA isolation

1. Add 20 µl of Oligo(dT)$_{25}$ Beads (Preparation of Oligo(dT)$_{25}$ Beads, page 12, step 6) to each tube containing cell lysate.
2. Rotate tubes slowly (approx. 5 rpm) for 10 min at room temperature on a device allowing both tilting and rotation.
3. Place the tubes into the AdnaMag-S without the magnet slider. Swing the AdnaMag-S downward to release beads and liquid captured in the cap.
4. Insert the magnet slider. After 1 min, transfer the supernatant containing the DNA into a new 1.5 ml tube (provided) and store at 4°C until use (for subsequent Genomic DNA Purification, page 14, step 15).
5. Wash with the RNA Purification Buffer A:
   - 5a. Remove the magnet slider from the AdnaMag-S.
   - 5b. Add 100 µl RNA Purification Buffer A to each tube and resuspend the beads by repeated pipetting. To avoid any loss of beads, rinse the lid and tube wall thoroughly.
   - 5c. Insert the magnet slider into the AdnaMag-S.
   - 5d. After 1 min, remove the supernatant completely.
   - 5e. Repeat steps 5a to 5d once (two washes in total).
6. Wash with the RNA Purification Buffer B:
   - 6a. Remove the magnet slider from the AdnaMag-S.
   - 6b. Add 100 µl RNA Purification Buffer B to each tube. Resuspend the beads by pipetting, and transfer into new 1.5 ml reaction tubes (provided).
   - 6c. Insert the magnet slider into the AdnaMag-S.
   - 6d. After 1 min, remove the supernatant completely. This step must be carried out carefully (watch the pellet) since the beads may slide and could be removed by mistake.
   - 6e. Repeat steps 6a to 6d once in the same reaction tubes (two washes in total).
7. Remove the magnet slider from the AdnaMag-S.
8. Add 100 µl ice-cold Tris·Cl Buffer to each tube, and resuspend the beads by pipetting.
9. Insert the magnet slider into the AdnaMag-S.

10. After 1 min, remove the supernatant completely.
11. Remove the magnet slider from the AdnaMag-S.
12. Depending on the downstream applications, follow one of the two options as follows.

    Option 1: for cDNA synthesis without magnetic beads (mRNA is removed from the beads by heating):

    Add 10–20 µl of cold 10 mM Tris·Cl. Heat to 80°C for 2 min and place the tube immediately on the magnet. Quickly transfer the eluted mRNA to a new RNase-free tube and continue with downstream application or store at –80°C.

    Option 2: cDNA can be generated using the Oligo-dT on the magnetic beads as primer (as performed in the AdnaTest procedure):

    Add 29.5 µl RNase-Free Water instead and follow the reverse transcription protocol using the SensiScript RT Kit in "Appendix: Reverse Transcription Using the Sensiscript RT Kit", page 18. Use the RNase-Free Water aliquot that was prepared from the 10 ml bottle of RNase-Free Water. For details, please see Important points before starting on page 11.

    Important: Do not store the mRNA/bead complex.

Genomic DNA purification

13. Prepare Elution Buffer AVE/APN. For each sample, mix 22.4 µl Buffer AVE with 7.6 µl Elution Buffer APN.

14. Add 600 µl RNase-Free Water to each tube containing cell lysate from step 4, page 12.
15. Add 40 µl Proteinase K to each sample, pulse-vortex 3 times, and incubate for 10 min at 56°C.
16. Add 150 µl Binding Buffer APN to each sample.
17. Add 30 µl Magnetic Bead Suspension APN to each sample.
18. Rotate tubes slowly (approx. 5 rpm) for 10 min at room temperature on a device allowing both tilting and rotation.
19. Place the tubes into the AdnaMag-S without the magnet slider. Swing the AdnaMag-S downward to release beads and liquid captured in the cap.

20. Insert the magnet slider. After 30 s, remove supernatant.

21. Wash with Buffer APN1:

    21a. Remove the magnet slider from the AdnaMag-S.

    21b. Add 500 µl Wash Buffer APN1 to each tube. Close the tubes and shake the AdnaMag-S gently back and forth 5 times to wash the magnetic beads.

    21c. Insert the magnet slider into the AdnaMag-S and invert the rack twice to release remaining beads from the cap.

    21d. After 30 s, remove the supernatant completely.

22. Wash with Buffer APN2:

    22a. Remove the magnet slider from the AdnaMag-S.

    22b. Add 500 µl Wash Buffer APN2 to each tube. Close the tubes and shake the AdnaMag-S gently back and forth 5 times to wash the magnetic beads.

    22c. Insert the magnet slider into the AdnaMag-S and invert the rack twice to release remaining beads from the cap.

    22d. After 30 s, remove the supernatant completely.

    22e. Repeat steps 22a to 22d once in the same reaction tubes (two washes in total).

23. Remove the magnet slider from the AdnaMag-S, remove the reaction tube, and centrifuge briefly.

24. Place the reaction tube back in to the AdnaMag-S rack and insert the magnet slider into the AdnaMag-S.

25. After 30 s, remove residual wash buffer completely.

26. Resuspend beads in 25 µl Elution Buffer AVE/APN (prepared in step 1) by repeated pipetting (5x) and incubate for 1 min at room temperature.

27. Centrifuge briefly.

28. Place the reaction tube in the AdnaMag-S and transfer the eluate into a new 1.5 ml tube.
29. Place reaction tubes with the gDNA on ice for subsequent analysis or store at –30 to –15°C.

Note: If gDNA is used in PCR downstream applications, performance may be improved by denaturing the gDNA eluate for 10 min at 95°C before adding the template to the reaction mix.

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 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 or protocols in this handbook (for contact information, visit **support.qiagen.com**).

		Comments and suggestions
Low yield		
a)	Lysis incomplete	The volume of cell-containing samples must be lower than 100 µl and preferably lower than 50 µl in order to guarantee optimal cell lysis with additional 200 µl AdnaTest lysis buffer.
b)	Wrong gDNA binding conditions	It is essential to strictly use 200 µl AdnaTest lysis buffer for cell lysis. Any other volume will lead to false binding conditions in the subsequent gDNA bead binding and, therefore, loss of gDNA.
c)	Wrong elution conditions	gDNA elution is pH dependent. Make sure that the elution buffer is freshly prepared and does not get longer time contact to air since the buffer pH may increase due to CO_2 content in the air and therefore lose its elution ability.
RNA degraded		
RNase contamination		Although all AllPrep buffers have been tested and are guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce any RNases during the AllPrep DNA/RNA procedure or later handling.
Low performance in downstream applications		
a)	Secondary structures	gDNA isolated in this workflow has high molecular weight and contains, therefore, secondary structures that may lead to problems with downstream applications like PCR or qPCR. If such a downstream procedure is necessary, proper denaturation of the gDNA (10 min heating at 95°C) is recommended. However, if the downstream application needs double-stranded DNA like NGS for instance, such a denaturation has to be avoided.
b)	gDNA loss during storage	If the gDNA eluates are stored at –80 to –20°C for longer than 1 week, denaturation may occur due to the high pH value of the gDNA elution buffer, which mimics lower gDNA concentrations using instruments like Qubit or Nanodrop. If such denaturation during storage shall be avoided, the addition of 1µl 100 mM Tris·Cl buffer pH7 is recommended.

Appendix: Reverse Transcription Using the Sensiscript RT Kit

Things to do before starting

Thaw 10x Buffer RT and dNTPs from the Sensiscript RT Kit (cat. nos. 205211 and 205213) at room temperature. Mix by vortexing. Centrifuge briefly and store on ice. Thaw RNase-Free Water (also from the Sensiscript RT Kit).

Adjust a thermal block or water bath to 50°C. Resuspend the Oligo(dT)$_{25}$ B.

Procedure

1. Prepare the RT master mix on ice. The RT master mix is prepared as shown in Table 1 according to the number of samples.

   The volume of the RT master mix should be 10% greater than calculated for the total number of reverse transcription reactions. A negative control reaction without addition of mRNA must always be prepared (RT control).

2. Vortex the RT master mix. Centrifuge briefly, and pipet 10.5 µl for each reaction into 0.2 ml PCR tubes.

3. Resuspend the mRNA/bead complexes (step 12, option 2, page 14) carefully with a pipette. Transfer the total volume into the 0.2 ml PCR reaction tube containing the RT master mix. Mix thoroughly by repeated pipetting.

Table 1. Reverse transcription reaction setup

Component	Volume
RT master mix	
10x Buffer RT	4.0 µl
dNTP Mix (5 mM each dNTP)	4.0 µl
RNase Inhibitor, 40 U/µl (Promega)	0.5 µl
Sensiscript Reverse Transcriptase	2.0 µl
Template RNA*	
mRNA/bead complex or RNase-Free Water	29.5 µl
Total volume	**40.0 µl**

* As RT control, add 29.5 µl RNase-Free Water instead of mRNA/bead-complex. The volume of the mRNA/bead-complex may vary slightly. In any case, use the total volume for reverse transcription.

4. cDNA is synthesized in a thermal cycler under the following conditions (Table 2).

Table 2. Reverse transcription program

Temperature	Time
37°C	60 min
93°C	5 min
4°C	∞

5. Place reaction tubes with the cDNA on ice for subsequent analysis or store at –20°C.

Ordering Information

Product	Contents	Cat. no.
AllPrep DNA/mRNA Nano	For 12 parallel isolations of mRNA and gDNA from low-biomass input samples	80272
Related products		
AdnaTest CTC-Select	For enrichment of tumor cells in whole blood for cancer research	395092
AdnaMag-S	Magnetic rack for 8 tubes, 1.5 ml	399911
Sensiscript RT Kit (50)*	For 50 reverse-transcription reactions: Sensiscript Reverse Transcriptase, 150 µl 10x Buffer RT, 100 µl dNTP Mix (contains 5 mM each dNTP), 1.1 ml RNase-Free Water	205211
UltraRun LongRange PCR Kit (100)	For 100 x 20 µl PCR reactions: master mix kit for ultrafast hot-start–mediated long-range PCR permitting moderate multiplexing	206442
UltraRun LongRange PCR Kit (500)	For 500 x 20 µl PCR reactions: master mix kit for ultrafast hot-start–mediated long-range PCR permitting moderate multiplexing	206444

Product	Contents	Cat. no.
Investigator Quantiplex Pro Kit (200)	For use on Applied Biosystems 7500 Real-Time Systems: Quantiplex Pro Reaction Mix, Quantiplex Pro Primer Mix, Quantiplex Pro Control DNA M1, QuantiTect® Nucleic Acid Dilution Buffer	387216
Investigator Quantiplex Pro RGQ Kit (200)	For use on QIAGEN RotorGene Q Real-Time Systems: Quantiplex Pro RGQ Reaction Mix, Quantiplex Pro RGQ Primer Mix, Male Control DNA M1, QuantiTect Nucleic Acid Dilution Buffer	387316

* The Sensiscript RT Kit (50) is sufficient for only 25 samples using AllPrep DNA/mRNA Nano because twice the volume is required for each reaction.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Document Revision History

Date	Changes
01/2021	Initial release
03/2021	Corrected the typographical error in step number (from “step 12a to 12d” to “step 22a to 22d”).

Limited License Agreement for AllPrep DNA/mRNA Nano Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

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Notes

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