



Mole[®]

MoleStrips[™] mRNA
Product No MG18-102

MGM-105-004

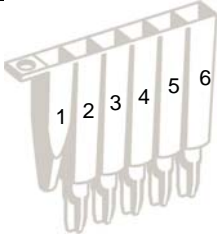
MoleStrips™ mRNA

Intended Use

MoleStrips™ mRNA is used together with the GeneMole® instrument to purify intact poly adenylated (polyA) mRNA directly from the lysate (using Mole mRNA lysis buffer) of animal and plant cells and tissues. The isolated mRNA is suitable for use in all downstream applications. The Oligo (dT)₁₄ beads and the buffers provided in the kit are produced and packed under RNase-free conditions. The Oligo (dT)₁₄ beads can bind up to 0.6 µg mRNA. This product is for research use only.

Materials Supplied

Prod. No.	MG18-102
No. of preps	64
Lysis Buffer	2 x 12 ml

Content of each well in the MoleStrips™ mRNA	 MoleStrip™ mRNA
1. Empty	
2. Magnetic Beads	
3. Conditioner Buffer	
4. Wash 1	
5. Wash 2	
6. Elution Buffer (10 mM Tris, pH 7.5)	

Additional Material Required

Product	Prod. No.
MoleTips	MG10-012
MoleTubes (non sterile/sterile)	MG10-013/MG10-014
MoleCaps	MG10-015
Disposable waste bins*	MG10-008
MoleTubes (homogenization beads)*	MG17-101-106
MoleMagnet*	MG10-009

*Optional. When working with RNA it is highly recommended to use disposable waste bins.

Recommended Input and lysis volume

	MoleStrips™ mRNA kit		
Lysis volume	150 µl	250 µl	370 µl
Cultured cells	<4 x 10 ⁵	<7 x 10 ⁵	<1 x 10 ⁶
Lysed tissue	Equivalent to cultured cells		
Pretreated whole blood			

Storage

The MoleStrips™ mRNA kit should be stored at 4°C and placed at room temperature one hour before use.

Starting Material

- Fresh cells or cells pelleted and stored at -70 °C prior to mRNA extraction.
- Fresh tissue, tissue stored in RNAlater or tissue frozen directly on dry ice or in liquid nitrogen and stored at -70 °C prior to mRNA extraction.

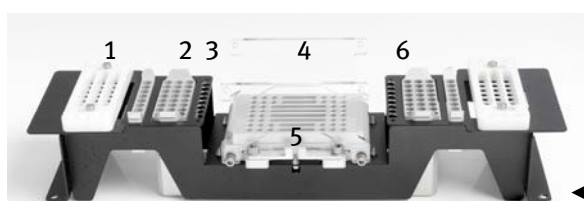
For mRNA extraction, GeneMole® can process up to 8 samples in one run.

Protocol

1. Switch on the GeneMole[®] instrument. Wait until the power indicator turns green (this may take 2 min).
2. Open the GeneMole[®] door and lift out the worktray.
3. Resuspend the MoleStrips[™] contents by placing well 2 of the MoleStrips[™] (contains beads) on a small vortex until the beads are resuspended. Open the jig handles (ref. 5 in Figure 1) and place the black adapter plate with the MoleStrips[™] in the jig. Fasten the MoleStrips[™] in the jig by closing the jig handles.

Important: Please make sure the black adapter plate is positioned between the jig and the MoleStrips[™] and ensure the MoleStrips[™] are pressed all the way down into position before locking the jig handles.

4. Using Figure 1 as a reference load the worktray with tips, tubes and MoleStrips[™] according to the number of samples to be processed. Add Tips (1), waste bin (2), collection tubes (6) and MoleStrips[™] (4) and then place the tray in the GeneMole[®].



Positioning holes indicate front of tray

Figure 1: Loading of the worktray for mRNA extraction;

1: pipette tips, 2: empty waste bin, 3: sample tubes, 4: adaptor plate and MoleStrips[™] 5: jig handles, 6: empty tubes.

5. Preparation of sample:

Step	Cell pellet	Tissue sample
A) Lysis	Add appropriate volume of lysis buffer (150, 250 or 370 μ l) to the cell pellet and mix 10 times with a pipette (set volume to 20 % less volume when mixing).	Add appropriate volume of lysis buffer to the tissue sample
B) Homogenization	<ol style="list-style-type: none"> 1. Pass the lysate through a 21-gauge needle with a syringe 10 times 2. Sonication 3. "bead-beater" with homogenization tubes (MoleTubes) - and proceed to step 6 below**. 	Homogenize the tissue sample*
C) Centrifugation	Centrifuge the lysate 30 sec at 2500 rpm to remove foaming and proceed to step 6 below.	Centrifuge the lysate 1 min at 5000 rpm and proceed to step 6 below.

*The method used for homogenization needs to be optimized for each specific tissue sample. Disruption of the tissue sample by homogenization is often performed using a polytron, a "bead-beater" (e.g. the Precellys 24 from Bertin Technologies) or by grinding the tissue with a mortar and pestle.

**No DNA shearing is necessary for cultured cells under 1×10^5

6. Transfer the lysates to GeneMole[®] sample tubes and place the tubes in the worktray according to Figure 1.
7. Place the worktray back into the GeneMole[®] and ensure it is correctly aligned by using the positioning pins located at the base of the instrument. Fit the pins into the holes located in each front corner of the worktray. Close the GeneMole[®] door.
8. Use the touch screen to select "Run A Protocol" from the GeneMole Menu. The Run Preparation screen will appear.

9. Select the protocol “**mRNA 8**”, from the dropdown menu and confirm your choice by pressing “**Accept**”. Specify the lysate volume, by activating the relevant window on the run preparation screen. Use the up and down buttons for scrolling.
10. Choose “**Next**”. The *Run Preparation* screen will appear. Verify correct loading of the worktray by pressing “**OK**”.
11. Start protocol run by pressing “**Start**”.
Note: The blinking green light located below the touch screen indicates that the GeneMole® is carrying out a protocol run.
12. The touch screen will display “Run Completed” and the instrument will generate a sound signal when the run is completed.
13. Upon completion of a run, open the GeneMole® door and collect the tubes at position 6 (see Figure 1) containing the collected oligo(dT) beads.
14. The mRNA can be manually eluted for further downstream work or the mRNA bound beads can be used directly to synthesize solid phase CDNA:
Manual elution: (i) Add the desired volume of elution buffer (well 6 in strip) to the tubes containing oligo(dT) beads linked to the target mRNA. (ii) Incubate at 65-80 °C for 5 minutes (remember to close tubes with caps). (iii) After incubation place directly on a MoleMagnet for 2 minutes. Transfer the eluate to clean tubes.
Solid phase CDNA synthesis: Follow step (i) and (ii) above, (iii) Place the tubes directly on ice, (iv) sample ready for solid phase cDNA synthesis. It is recommended to use 0.1 µl or less cDNA in PCR applications.

Cleaning procedures

Perform cleaning procedures if necessary after a GeneMole® run. It is recommended to clean relevant instrument parts with RNase AWAY wipes between runs when performing mRNA extractions. For more detailed cleaning and maintaining instructions please refer to the GeneMole® User Manual.

Safety Information

When working with chemicals always wear protective gear. For more information, please consult the appropriate material safety data sheets. The MSDS is available upon request.

Product Warranty and Satisfaction Guarantee

Mole Genetics 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. Product warranty limits Mole Genetics liability only to the cost of the product.

For further information about GeneMole® and available applications see
www.molegenetics.com and www.molecookbook.com

The logo for Mole Genetics, featuring the word "Mole" in a bold, orange, sans-serif font with a registered trademark symbol (®) to the upper right. The logo is centered on the page and is surrounded by four black dots: one above, one to the right, one below, and one to the left, arranged in a square pattern.