Taiclone®
Quality Guarantee

USER MANUAL



PURIFICATION KIT

ClonMag™ mRNA Purification Kit

Catalog No.tcpk2991 1 Kit

FOR RESEACH USE ONLY!

Please read completely user manual and storage condition.

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ClonMag™ mRNA Purification Kit

Catalog No.tcpk2991

Available Sizes

Size: 1 Kit

Specifications

Application: For Purification of mRNA with magnetic separation.

ClonMag™ mRNA Purification Kit is designed specifically for the isolation of pure, intact mRNA from purified total RNA utilizing magnetic separation technology.
The isolation takes 15 minutes or less.
The kit contains all the reagents necessary to isolate a typical range of 30-65µg of high quality mRNA in a single round of isolation. Increased yields are obtained from pooling multiple rounds of mRNA isolated from the same sample, using the same particles.

ClonMag™ mRNA Purification contains 1ml (10mg) of ClonMag™ Stepavidin Biotinylated Oligo $(dT)_{25}$ Complex. is an aqueous suspension of 5 micrometer diameter, 50 nanometer pore diameter, superparamagnetics, totally porous glass particles with strepavidin covalently coupled to its surface and preloaded with Biotinylated Oligo (dT)25 at a level optimized for maximal mRNA yield.

This Purification Kit contains all reagents necessary to isolate and purify from 30 - 70 ug of high quality mRNA (using mouse liver total RNA) from single round of isolation.

Storage.

Store kit components at 4 °C .
Long term storage at - 70 °C
DO NOT USE past kit expiration date.

Components

1 ml ClonMag™ Strepavidin Biotinylated Oligo (dT)25 complex
(10mg, suspended in 50nM Tris HCL, pH 7.2, 2.0M NaCl,0.02% NaN3)
5 ml 2X Hybridization Binding Buffer
15 ml Hybridization Wash Buffer
1 ml Release Solution

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Materials needed (NOT Supplied)

Total RNA of Interest
Strong Magnetic Separator.
65 °C Water bath
UV/Vis Spectophotometer
Nuclease-free Purified water
1.5 ml Nuclease-free Microcentrifuge tubes
Vortex Mixer
Nuclease-free Pipettes and Pipettes Tips
Low Speed Rotator

IMPORTANT INFORMATION BEFORE YOU START YOUR PROCEDURES:

Using 1mg ClonMag™ Strepavidin Biotinylated Complex.
1 mg of ClonMag™ Strepavidin Biotinylated Complex can bind an average of 5ug of mRNA.

NOTE: The proportion of mRNA to Total RNA varies widely in different tissue.
mRNA constitues about 2% of Total RNA Isolated from Mouse liver.
Therefore to isolate 5ug of mRNA, 250ug of Total RNA (Isolated from mouse liver) per 1mg of ClonMag™ Strepavidin Biotinylated Complex is recommended)

THIS PROTOCOL CAN BE SCALED UP OR DOWN BY PROPORTIONALLY ADJUSTING THE COMPONENT VOLUMES.

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Isolation of mRNA from Total RNA procedure

1. Warm the kit component to room temperature
2. Vortex ClonMag™ Strepavidin Biotinylated Complex to fully suspen the particles.
3. Transfer 100ul (1mg) of ClonMag™ Strepavidin Biotinylated Complex to 1.5ml nuclease-free microcentrifuge.
4. Magnetically separated using a magnetic particle separator and carefully remove supernatant.
5. Resuspend ClonMag™ Strepavidin Biotinylated Complex in 2X Hybridization Binding Buffer (350ul per mg ClonMag™ Strepavidin Biotinylated Complex) and put aside until ready for hybridization.
7. Add 250 ug of Total RNA to a clean nuclease-free microcentrifuge tube.
8. Bring the volume to 350 ul with nuclease-free water.
   The final concentration of Total RNA should not exceed 0.75 ug/ul
9. Disrupt the secondary structure of the total RNA by heating at 65°C for 2-3 minutes.
10. Transfer the heat disrupted total RNA to the tube containing ClonMag™ Strepavidin Biotinylated Complex. Vortex and incubate 1-3 minutes in room temperature on a low speed rotator. Magnetically separate and carefully remive the supernatant.
11. Resuspend the mRNA-bound ClonMag™ Strepavidin Biotinylated Complex in Hybridaization Wasjh Buffer (350ul per mg ClonMag™ Strepavidin Biotinylated Complex). Magnetically separate and carefully remove the supernatant. Repeat two more times.
12. Resuspend the mRNA-bounded ClonMag™ Strepavidin Biotinylated Complex in Release solution (20ul per mg ClonMag™ Strepavidin Biotinylated Complex) and heat at 65°C for 2 minutes. Magnetically separate and carefully transfer the supernatant (which now contained isolated mRNA) to a new 1.5 ml nuclease-free microcentrifuge tube.
13. ClonMag™ Strepavidin Biotinylated Complex may be used repeatedly for multiple rounds of isolation from the same total RNA.
14. Resuspend the particles in 2 X Hybridization Binding Buffer and follow Step 5 through 12.
15. Pool all resulting of supernants.

Resulting of Yield and Purity of mRNA

1. Measure the Optical density (OD) of the isolated mRNA at wavelength of 260nm and 280nm.
   (NOTE: it is recommended to use TE Buffer to read OD.
   DO NOT use DEPC treated water to read OD.
   It will lower the A_{260}/A_{280} ratio by 0.2 - 0.3)

2. Yield od mRNA (ug/ml) = $(OD_{260})(40)$(Dilution factor)
   Purity of mRNA (ug/ml) = (OD_{260}/OD_{280})

NOTE (OD260)/(OD280) of Pure mRNA ù 2.0

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