

## PRODUCT INFORMATION

### DIRECTIONS FOR USE Mag-Net™, mRNA Isolation from Total RNA Kit (K269-25RXN)

#### Product Specifications

* Purification Method:	Oligo dT Magnetic Matrix
* Starting Material:	75 µg of Total RNA per Prep
* Preparation Time:	<15 Minutes
* Theoretical Yield:	30 µg of mRNA per Kit
* Number of Reactions:	25 per Kit

#### Kit Components

Mag-Net Oligo dT Matrix	2.5 ml
Binding Reagent	7.5 ml
Wash Reagent	10 ml
Elution Reagent	0.5 ml
Sterile Water	2.5 ml

#### 1. *Washing of Mag-Net™ Oligo dT Matrix;*

Add 100 µl resuspended Mag-Net Oligo dT Matrix to a sterile RNase-free microcentrifuge tube. Place the tube in the Mag-Net™ Tube Stand for 30 seconds and remove the clear supernatant. Remove the tube from the Mag-Net Tube Stand, add 100 µl of the Binding Reagent, and gently vortex to resuspend. Leave the Mag-Net Oligo dT Matrix resuspended until the Total RNA sample is prepared.

#### 2. *Preparation of the Total RNA Sample:*

Use 75 µg of Total RNA in a final sample volume of 100 µl to achieve optimal annealing between the mRNA and the Mag-Net Oligo dT Matrix. If a sample is more concentrated than 75 µg/100 µl, use nuclease-free water for dilution up to 100 µl. For a sample that is more dilute than 75 µg/100 µl, simply follow the protocol without modifying the sample. Add 100 µl of Binding Reagent to the Total RNA sample and incubate at 65°C for 2 minutes.

#### 3. *mRNA Isolation Protocol:*

Remove the Binding Reagent from the pre-washed Mag-Net Oligo dT Matrix (Step 1) by inserting the tube in the Mag-Net Tube Stand for 30 seconds. Remove the supernatant and add 100 µl of Binding Reagent.

#### 4. Add the 200 µl of Total RNA sample (Step 2) to the Oligo dT Matrix resuspension.

Mix the tube by inversion or rotation for 3-5 minutes at room temperature to allow annealing. Place the tube in the Mag-Net Tube Stand for 30 seconds and carefully remove the supernatant.

#### 5. Wash the magnetic matrix with 200 µl of the Wash Reagent, mix thoroughly, and carefully remove all traces of the supernatant using the Mag-Net Tube Stand. Repeat a second time.

#### 6. Add 20 µl of Elution Reagent to the tube, gently vortex, and pulse centrifuge to pool the matrix suspension to the bottom of the tube. Incubate at 65°C for 2 minutes. Place the tube in the Mag-Net Tube Stand for 30 seconds, then transfer the supernatant containing the mRNA to a sterile, RNase-free microcentrifuge tube. The final elution step may also be performed using sterile, deionized water or loading buffer containing formamide.

