7. Analyzing Results

7.A. **mRNA Yield**

Because mRNA is only 1-5% of total RNA in mammalian cells, 1-5 µg yield can generally be expected from 100 µg of starting total RNA. Micro–fluorometers or micro–spectrophotometers are recommended for determining these quantities. For UV absorbance methods blanked against the **mRNA Elution Solution**, measure the A_{260} (RNA absorbance) reading and use the following equation:

mRNA yield (µg) = A_{260} reading x dilution factor x 40 µg/mL x sample elution volume (mL)

For mRNA, an A_{260} of 1.0 = 40 µg/mL when measured in a cuvette with a 10 mm optical path length. In some cases subtracting the turbidity/cuvette impurity absorbance at A_{320} from the A_{260} reading may be necessary for a corrected reading that does not overestimate the mRNA quantity.

7.B. **mRNA Quality**

Similarly, mRNA quality can be estimated by different methods. With a micro–spectrophotometer, measure an additional absorbance at A_{280}. Highly pure mRNA has an A_{260}/A_{280} ratio of >1.8, indicating it has minimal contamination by proteins. Again, in some cases subtracting the turbidity/cuvette impurity absorbance at A_{320} from the A_{260} and A_{280} readings may be necessary. **Figure 2** below displays a spectrophotometric curve of mRNA purified from human HEK293 cells using the **AbraMag® mRNA Magnetic Purification Kit**.

Agarose gel electrophoresis is also recommended to evaluate quality. The flowthrough after the mRNA is bound to the oligo (Section 6.C.4) can be run on a gel next to a portion of the eluted sample to ensure that rRNA was completely removed in the flowthrough. **Figure 3** below shows an agarose gel electrophoresis analysis of mRNA purified from HEK293 cells using the **AbraMag® mRNA Magnetic Purification Kit**. Note the total removal of rRNA banding in the elution.

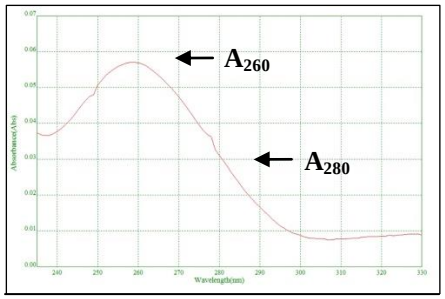

Figure 2. Spectrophotometric analysis of mRNA.

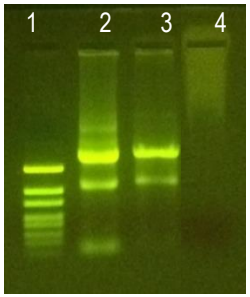

Figure 3. Gel electrophoresis analysis of mRNA.
Lane 1-ladder; 2-total HEK293 RNA; 3-flowthrough; 4-eluted mRNA

The AbraMag® mRNA Magnetic Purification Kit is intended for research and *in vitro* use only. This product was not tested or certified for diagnostic use.

General Limited Warranty: Abraxis Inc. warrants the products manufactured by the Company, against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. **Abraxis Inc. makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.**

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AbraMag® mRNA Magnetic Purification Kit

For mRNA Purification from Total RNA

Product No 555025 (10 purifications)

1. General Description

The **AbraMag® mRNA Magnetic Purification Kit** is designed to purify mRNA out of total RNA. Paramagnetic carboxyl beads with uniform particle size, bound with oligo (dT)25, efficiently base pair with poly-A-mRNA, resulting in high yields of mRNA without ribosomal RNA (rRNA) carryover. The kit is intended for manual purifications using a magnetic separator.

2. Safety Instructions

Always use appropriate protective equipment (including but not limited to gloves, lab coats, and safety glasses) when working with RNA. The magnetic beads solution contains sodium azide as a preservative, which may react with lead or copper plumbing to produce metal azides that might cause explosion. To prevent azide accumulation in plumbing, flush with copious amounts of water immediately after disposal. Refer to Safety Data Sheet for further information.

3. Storage and Stability

Upon delivery of the kit, remove the **AbraMag® mRNA Purification Magnetic Beads** and store at 4°C. **Do not freeze the magnetic beads solution**. All other kit reagents may be stored at room temperature (20-25°C). Do not use after the printed expiration date.

4. Kit Principle

The **AbraMag® mRNA Magnetic Purification Kit** process uses a simple, efficient, magnetic bead-based procedure for mRNA purification from total RNA, as illustrated below in **Figure 1**:

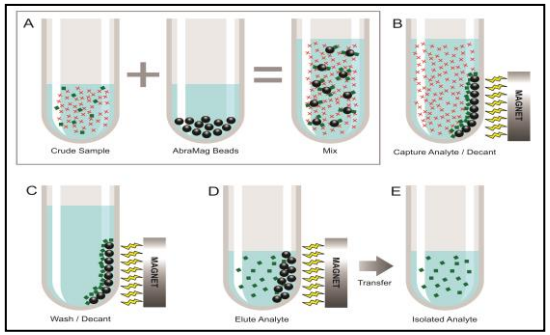

Figure 1. Schematic of the **AbraMag® mRNA Magnetic Purification Kit** process.

4.A. **Mixing:** The total RNA sample is added to washed AbraMag® mRNA purification magnetic beads.

4.B. **Binding:** Poly-A mRNA binds to the oligo (dT)-coupled beads in the presence of the Binding Solution. A magnet is used to secure the beads, with mRNA attached.

4.C. **Washing:** Remaining rRNA and other non-targets are washed away in a series of two wash steps.

4.D. **Elution:** mRNA is then eluted and transferred to a new tube.

4.E. **Downstream Applications:** Purified, high-quality isolated mRNA may then be used for downstream procedures such as RT-PCR and qRT-PCR, or stored long-term.

5. Limitations and Precautions

Initial handling of total RNA can significantly affect the yield and quality of resulting mRNA. To avoid degrading the total RNA, keep samples frozen at -80°C in nuclease-free water until purification. Avoid freezing and thawing samples repeatedly. Overall mRNA yield, quality and test reproducibility may vary depending on amount, age, and condition of total RNA before and after storage. Input sample RNA should be evaluated by gel, spectrophotometer, fluorometer, and/or bioanalyzer methods to confirm quality and quantity before beginning mRNA purification.

Ribonucleases (RNases) are highly stable enzymes that degrade RNA. **Sample contamination with RNases will result in partial or complete degradation of total RNA and mRNA.** To minimize sources of RNase contamination, use RNase-free disposable microcentrifuge tubes and pipette tips with filter barriers. Always wear disposable gloves and change them frequently. Clean pipettes and lab benchtops with 10% bleach and/or RNase-degrading solutions before beginning the procedure. All kit solutions are provided RNase-free in diethyl pyrocarbonate (DEPC)–treated water; do not leave solutions uncapped longer than necessary to prevent their becoming contaminated with environmental RNases.

Before each use, check for any precipitate formation in the solutions. If observed, shake to re-dissolve any precipitates.

6. Working Instructions

6.A. **Materials Provided**

1. DEPC–treated water, 2 x 1mL
2. AbraMag® mRNA Purification Magnetic Beads, 0.5 mL
3. mRNA Binding Solution, 4 mL
4. mRNA Wash Solution, 3 mL
5. mRNA Elution Solution, 1 mL

6.B. **Additional Materials and Equipment Required** (not included with the kit)

1. Total RNA, ~50 to 125 µg, pelleted or in nuclease-free water
2. Disposable gloves and other protective equipment
3. Micro-pipettes with disposable plastic filter barrier tips
4. 1.5 mL nuclease-free microcentrifuge tubes
5. 4°C refrigerator
6. Heating block, thermomixer, or water bath capable of 65°C
7. Ice
8. Magnetic microcentrifuge tube separator, Solo (Abraxis PN 472270) or Multi-6 (Abraxis PN 472260) or similar
9. Vortexer

6.C. **Kit Procedure**

1. Resuspend or bring the total RNA sample volume to 100 µL in a 1.5 mL sterile, nuclease-free microcentrifuge tube using the provided **DEPC-treated water**. Incubate the sample at 65°C for 5 minutes. Remove and transfer the tube directly to ice.

2. During the incubation, prepare the beads:

   a. Vortex the AbraMag® **mRNA Purification Magnetic Beads** with ~1 second pulses, ensuring uniform suspension of the beads.

   b. Add 50 µL of beads solution to a nuclease-free microcentrifuge tube. Place the tube on a magnetic separator for ~30 seconds. Leaving the tube on the separator, remove and discard the supernatant using a pipette, without disturbing the beads that have collected at the magnet.

   c. Remove the tube from the separator and add 100 µL **mRNA Binding Solution**. Pipette gently to mix. Return the tube to the separator for ~30 seconds. Leaving the tube on the separator, remove and discard the supernatant using a pipette, without disturbing the beads that have collected at the magnet.

   d. Repeat step 2.c.

   e. Resuspend the beads in 100 µL **mRNA Binding Solution** by pipetting.

3. Add total RNA prepared in Section 6.C.1 (100 µL) to beads, resulting in a 1:1 ratio of sample and **mRNA Binding Solution**. Pipette gently to mix. Incubate at room temperature for 5 minutes to allow mRNA to base pair with the oligo (dT) on the bead surface.

4. Place the tube on a magnetic separator for 1 minute. Leaving the tube on the separator, remove and discard the supernatant using a pipette, without disturbing the beads collected at the magnet.
   Note: The flowthrough may be saved for gel analysis. See Section 7.B.

5. Remove the tube from the separator and add 100 µL **mRNA Wash Solution**. Pipette gently to mix. Return the tube to the separator for 1 minute. Leaving the tube on the separator, remove and discard the supernatant using a pipette, without disturbing the beads collected at the magnet.

6. Repeat step 5.

7. Add 50 µL **mRNA Elution Solution** to the beads. Pipette gently to mix. Incubate the sample at 65°C for 2 minutes.

8. Vortex the solution once with a ~1 second pulse to ensure the sample is mixed well. Return the tube to the magnetic separator for 1 minute. Leaving the tube on the separator, transfer the eluate to a new microcentrifuge tube using a pipette. ***The eluate contains the purified mRNA.***

6.D. **Storing mRNA**

Store the purified mRNA in the **mRNA Elution Solution** on ice for immediate use, or at -80°C for long-term storage. To avoid repeated freezing and thawing, consider storing the mRNA in aliquots.