I. Introduction

This protocol is used with the PrepX PolyA mRNA Isolation Kit, 96 Samples (Cat. No. 640098), which accommodates rapid, walkaway automation of mRNA isolation on the Apollo™ Library Prep System. When run with the `PrepX PolyA 48` script, this kit can be used to process **up to 48 samples per batch**. **Read this Protocol-At-A-Glance in its entirety before you begin, with particular attention paid to the Apollo System Best Practices.**

II. Workflow Overview

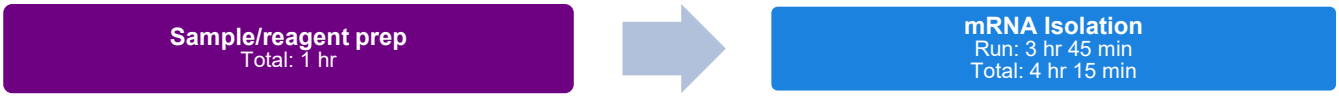


Figure 1. PolyA mRNA isolation workflow overview for one batch of 48 samples on the Apollo system with the PrepX PolyA mRNA Isolation Kit, 96 Samples. Blue and purple boxes indicate steps performed on and off the Apollo system, respectively. Run = the run time in minutes on the Apollo system, if applicable. Total = total time in minutes spent, including thawing of reagents, reagent and equipment setup, heating and cooling of thermal blocks, incubation of reactions, and automated liquid-handling processes, if applicable.

III. Materials Required

Input material

100 ng–1 µg of total RNA in a 50 µl volume (concentration of 2 ng/µl–20 ng/µl).

NOTE: RNA with an RNA integrity number (RIN) of less than 9.0 will not give optimal results when used with these protocols.

List of Components

PrepX PolyA mRNA Isolation Kit, 96 Samples (400047) Store at –20°C.	640098
Reagent 1–RNA Binding Buffer, 2x	56.7 ml
Reagent 2–Wash Buffer	65.1 ml
Reagent 3–Elution Buffer	25.2 ml
Reagent 4–Oligo-dT Beads	1.814 ml

Additional Materials Required

The following consumables from Takara Bio were used to validate protocols and scripts. **Do not make any substitutions.**

Apollo consumables	Cat. No.	Quantity	Usage/48-rxn run
Apollo Filter Tips	640084	Box of 960 tips	152
Apollo Reservoirs	640087	Box of 100 reservoirs	4 reservoirs
Apollo Microtiter Plates	640083	Box of 25 plates	2 plates
Apollo 1.1 mL MiniTubes	640088	Box of 960 minitubes	96 tubes
Apollo 0.2 ml PCR 8-tube strips, Clear	640082	Box of 125 strips	24 strips
Apollo Caps for 0.2 ml PCR 8-Tube Strips, Clear	640086	Box of 125 strips	12 strips

General lab equipment, reagents, and consumables

- Single-channel pipettes: 10 µl, 20 µl, 200 µl, and 1,000 µl
- Eight-channel pipettes (recommended): 20 µl and 200 µl
- Filter pipette tips: 2 µl, 20 µl, 200 µl, and 1,000 µl
- PCR thermal cycler
- 100% ethanol (EtOH; molecular biology grade)

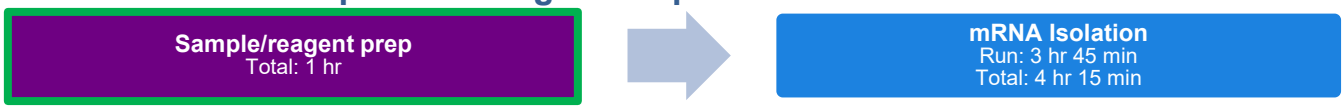
IV. Apollo System Best Practices

- **Read this Protocol-At-A-Glance in its entirety before you begin.**
- Clean the work surfaces, including the retention plates, with 70% ethanol at least once a week and immediately prior to this protocol.

  NOTE: While the standard cleaning procedure is sufficient to clean Apollo system surfaces for RNA work, the user may wish to use additional decontamination solutions to remove nucleases. As these are known to be corrosive and may damage the system, ensure any nuclease decontamination is immediately followed by a cleanup with 70% ethanol.
- **Restart the instrument before every run. Also, between each subprotocol, perform a power cycle by turning the instrument off, waiting 1 min, and then turning it back on.**
- Discard any deformed plastics.
- Separate partial tube strips with scissors and remove resulting plastic overhangs.
- Spin down reagents before placing them on the deck to avoid air bubbles. **Bubbles at the bottoms of tubes must be removed to ensure accurate volume delivery.**
- Ensure plastics are properly seated on the deck surface **with caps/lids removed**. Be sure to push any tubes down completely and evenly prior to installing the metal retention plates.
- Empty the waste box before every run. **An accumulation of tips in the waste box may cause the run to fail.**

V. Protocols

A. Protocol: Sample and Reagent Prep



*For each protocol, the corresponding step in the workflow diagram is indicated in green.

Materials Required

Reagents	Storage conditions	Source
Sample (RNA)	–80°C	User

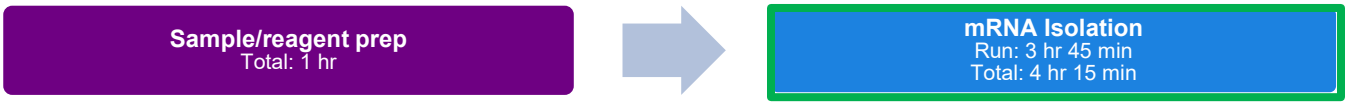
Apollo consumables	Source	Cat. No.	Quantity	Usage/48-rxn run
Apollo 0.2 ml PCR 8-tube Strips, Clear	Takara Bio	640082	Box of 125 strips	32 strips
Apollo Caps for 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640086	Box of 125 caps	32 strips

1. For each sample, prepare 100 ng–1 µg of total RNA in a 50-µl volume (for a concentration of 2–20 ng/µl).

   NOTE: We recommend QC of RNA samples with a Bioanalyzer instrument (Agilent Genomics) prior to use with this kit. RNA with an RNA integrity number (RIN) <9.0 will not give optimal results when used with these protocols.
2. Prepare 32 strips of 6-well tubes by cutting off 2 tubes from the end of each strip and trimming off excess plastic.

   NOTE: The system is calibrated for Apollo PCR tubes **only. Using other tubes may cause the run to fail.**
3. On the benchtop, dispense the total RNA sample into the Apollo 0.2 ml PCR 8-tube Strips in 50-µl aliquots.

B. Protocol: mRNA Isolation



Materials Required

Reagents	Storage conditions	Source
Sample (RNA)	4°C	Section V.A.
Reagent 1–RNA Binding Buffer, 2x	4°C	Takara Bio
Reagent 2–Wash Buffer	4°C	Takara Bio
Reagent 3–Elution Buffer	4°C	Takara Bio
Reagent 4–Oligo-dT Beads	4°C	Takara Bio

Apollo consumables	Cat. No.	Quantity	Usage/8-rxn run
Apollo Piercing Tips	640085	Box of 1,000 tips	8 tips
Apollo Filter Tips	640084	Box of 960 tips	32 tips
Apollo 0.2 ml PCR 8-Tube Strips, Clear	640082	Box of 125 strips	6 strips

1. Turn on the instrument or, if the instrument is already on, perform a power cycle by turning the instrument off, waiting 1 min, and then turning it back on.
2. Press **Sample Prep** > **PrepX PolyA 48**. The **Cooling** indicator will appear.
3. Load consumables onto the Apollo system work surface according to the layout in Figure 2. First, load the consumables that do not initially hold reagents (table above). Just before the run, load the consumables containing reagents, but not samples, onto the system.
4. Wait until the **Cooling** indicator has disappeared, and the **Run** button has appeared, then load the samples.

   NOTE: Ensure plastics are properly seated on the deck surface with caps carefully removed. Be sure to push any tubes down completely and evenly prior to installing the metal retention plates.
5. Install the metal retention plates on Blocks 3 and 4.
6. Empty the waste box and remove any used consumables from the system.

   NOTE: An accumulation of tips in the waste box may cause the run to fail.
7. Close the instrument door and press **Run.**

   NOTE: The run time is 3 hours and 45 minutes.
8. When the run is complete, remove the PolyA mRNA products from Block 3, Rows 1–6, and put them on ice. The final mRNA product volume should be ~17 µl per tube.
9. Quantify the mRNA using the appropriate method or continue immediately to the appropriate PrepX mRNA protocol. Be sure to use the entire output volume for library preparation.
10. Turn off the instrument.

PrepX™ PolyA 48 Protocol

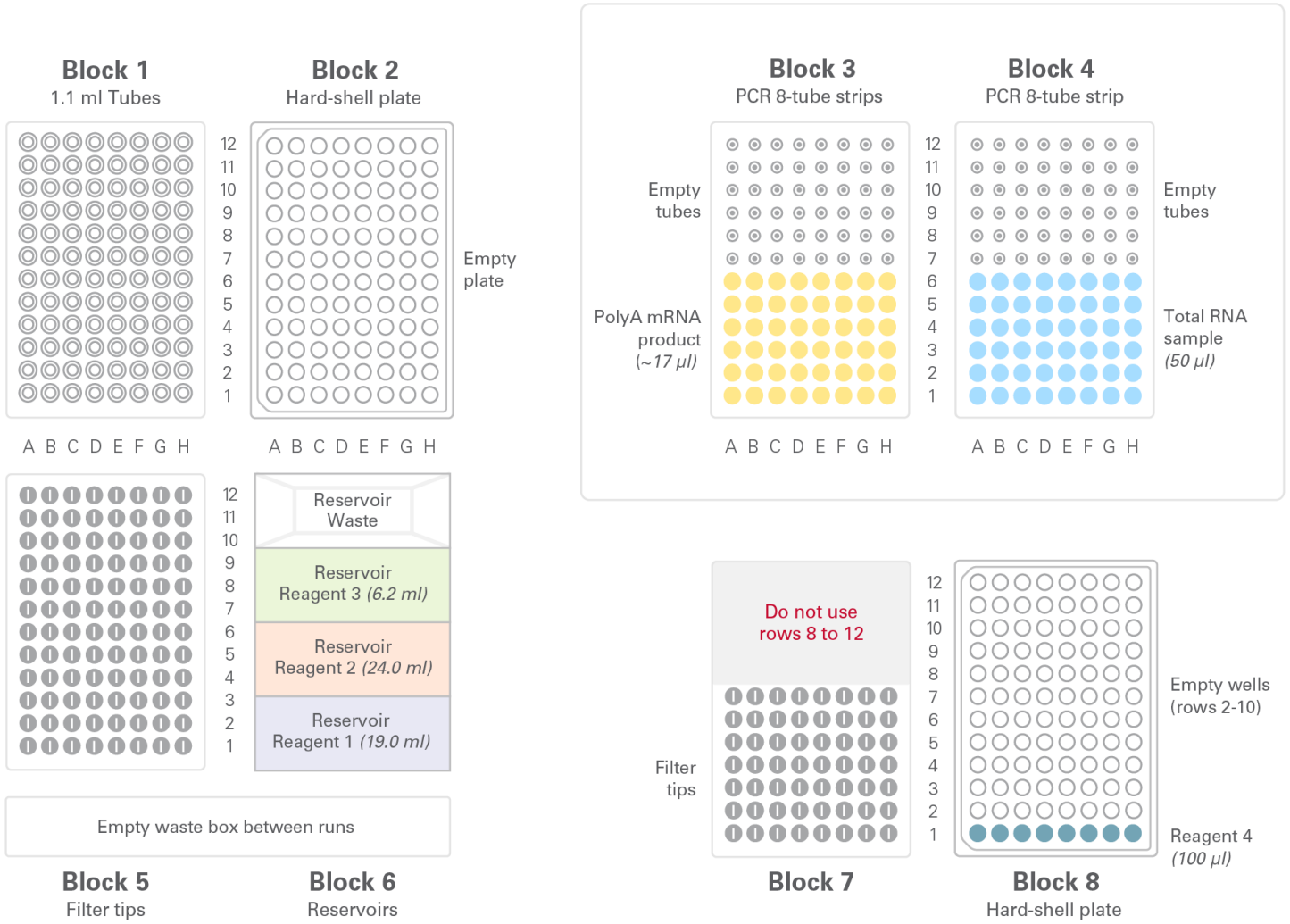


Figure 2. Deck layout for PolyA mRNA isolation from 48 samples. For this protocol, use script `PrepX PolyA 48`. Run time is 3 hours and 45 minutes.

Appendix A. PolyA mRNA analysis

Figure 3 demonstrates Bioanalyzer traces from isolated polyA mRNA samples on an Agilent RNA 6000 Pico Chip using 1 µg or 0.1 µg of human brain total RNA.

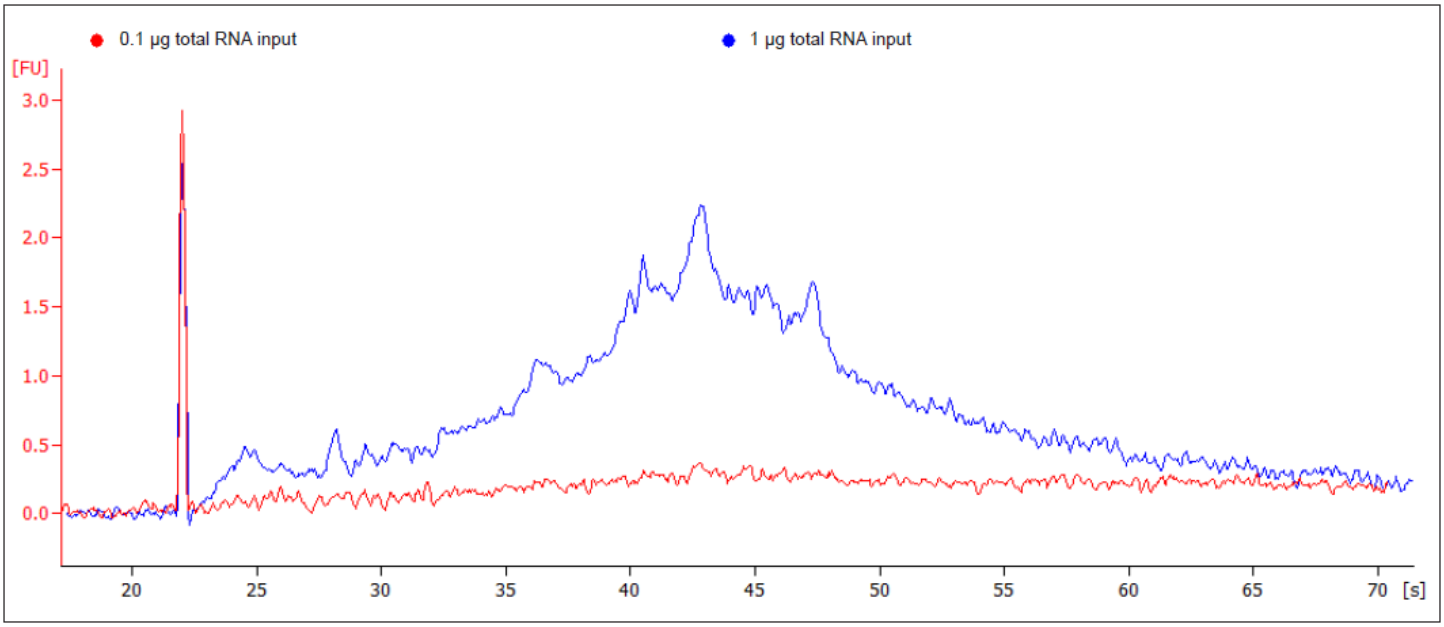


Figure 3. Bioanalyzer traces of isolated polyA mRNA samples. Human brain total RNA was processed through the PrepX PolyA mRNA Isolation Kit protocol and analyzed on an Agilent 2100 Bioanalyzer with Agilent's RNA 6000 Pico Kit using 1 µg or 0.1 µg of human brain total RNA.

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