

THE INSTITUTE FOR GENOMIC RESEARCH
Standard Operating Procedure

TITLE: **POLY A⁺ RNA ISOLATION FROM TOTAL RNA BY DYNABEADS®
OLIGO (dT)₂₅**

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SOP #: M014

REVISION LEVEL: .2

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AUTHOR: Linda Moy

PRIMARY REVIEWER: Fenglong Liu

1. PURPOSE

This protocol describes the isolation of poly A⁺ RNA from total RNA suspended in RNase-free water using Dynabeads® Oligo (dT)₂₅.

2. SCOPE

The procedure is used by the Human, Mouse, and Arabidopsis Microarray projects under the supervision of John Quackenbush within the Mammalian Genomics Department.

3. MATERIALS

- 3.1 Dynabeads® Oligo (dT)₂₅ (DynaL Biotech; Cat# 610.05)
- 3.2 EDTA (Sigma; Cat# E2628-2)
- 3.3 Lithium Chloride (Sigma; Cat# L7026)
- 3.4 Tris-HCl pH 7.5 (Sigma; Cat# 93372)
- 3.5 DEPC-Treated Water (Ambion; Cat#9920)
- 3.6 Dry Bath Incubator and 1.7 mL Heating Block (Fisher Scientific; Cat# 11718 and 11781-9)
- 3.7 Magnetic Particle Concentrator (MPC), 1.7 mL (DynaL Biotech; Cat# 120.20)
- 3.8 Microcentrifuge (Eppendorf; Model# 5415D)
- 3.9 Pipette Tips, 20 µL
- 3.10 Pipette Tips, 200 µL
- 3.11 Tubes, 1.7 mL
- 3.12 Vortex

4. REAGENT AND EQUIPMENT PREPARATION

4.1 Binding Buffer

- 4.1.1 Prepare 50 mL of binding buffer with the following final concentrations: 20 mM Tris-HCl at pH 7.5, 1 M LiCl, and 2 mM EDTA. Use RNase-free water.

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4.1.2 Bring to room temperature prior to use. Store the remaining portion at 4° C.

4.2 Wash Buffer

4.2.1 Prepare 50 mL of wash buffer with the following final concentrations: 10 mM Tris-HCl at pH 7.5, 0.15 M LiCl, and 1 mM EDTA. Use RNase-free water.

4.2.2 Bring to room temperature prior to use. Store the remaining portion at 4° C.

4.3 Elution Buffer

4.3.1 Prepare 15 mL of elution buffer with the following final concentration: 10 mM Tris-HCl at pH 7.5. Use RNase-free water.

4.3.2 Keep buffer on ice just prior and during use. Store the remaining portion at 4° C.

4.4 Dry Bath Incubators

4.4.1 Adjust one dry bath incubator to 70° C.

4.4.2 Adjust another dry bath incubator to 85° C.

5. PROCEDURE

5.1. Bead Preparation

NOTE: According to the manufacturer, “Do not leave the Dynabeads® Oligo (dT)₂₅ unsuspended for a long period of time, as drying of the Dynabeads® Oligo (dT)₂₅ may lower their [binding] capacity.”

5.1.1 Vortex Dynabeads® to resuspend. Be sure that the suspension is a uniform brown color.

5.1.2 Transfer 200 µL of bead suspension per 100 µg of total RNA into a 1.7 mL tube. For example, 200ug of total RNA requires 400uL of bead suspension.

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- 5.1.3 Place the tube in the Magnetic Particle Concentrator (MPC) for 1 minute. Check for good separation of beads and solution.
- 5.1.4 Remove as much of the storage buffer as possible from the tube by pipette without disturbing the pellet. Discard storage buffer.
- 5.1.5 Remove tube from MPC and completely resuspend the beads in 500 μ L of binding buffer. Mix by pipetting. Set aside until step 5.2.2.

5.2 poly A⁺ RNA Isolation

- 5.2.1 Mix an equal volume of binding buffer and total RNA in a 1.7 mL tube. For example, for a 500 μ L aliquot of total RNA, add 500 μ L of binding buffer. Mix well by pipetting.
- 5.2.2 Incubate total RNA and binding buffer mixture at 70° C for 5 minutes. During the last minute of the incubation period, place the tube with the re-suspended beads (from step 5.1.5) in the MPC.
- 5.2.3 After the 5 minute incubation period, remove and discard the binding buffer from the tube in the MPC.
- 5.2.4 Remove tube from the MPC and re-suspend the beads with the heated total RNA and binding buffer mixture. Mix well by pipetting.
- 5.2.5 Leave at room temperature for 15 minutes to allow the poly A⁺ RNA to hybridize to the beads.
- 5.2.6 Place tube in the MPC for 2 minutes.
- 5.2.7 Transfer buffer-sample mixture to a sterile tube, store at room temperature, and set aside for later re-extraction of any remaining poly A⁺ RNA.
- 5.2.8 Remove tube with beads from MPC. Resuspend beads in 500 μ L of wash buffer. Mix by pipetting.

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5.2.9 Place tube in MPC for 1 minute and discard wash buffer without disturbing the pellet.

5.2.10 Repeat wash (steps 5.2.5 and 5.2.6).

5.2.11 Resuspend beads in at least 40 μ L of elution buffer per 100 μ g of total RNA.

5.2.12 Incubate tube at 85° C for 2 minutes.

NOTE: The following two steps must be performed as quickly as possible to prevent RNA degradation and maintain Dynabead® Oligo (dT)₂₅ integrity. (Please refer to note in 5.1.)

5.2.13 Immediately place tube in MPC and quickly transfer the elution to a sterile tube without disturbing the pellet. The elution is the poly A⁺ RNA. Place poly A⁺ RNA on dry ice.

5.2.14 Immediately resuspend beads in 500 μ L of binding buffer and mix.

5.3 Bead Preparation for Re-Extraction

NOTE: Additional extractions may be performed to isolate any remaining poly A⁺ RNA from the sample. This may be desirable if total RNA concentration is relatively high. Usually only 1 round of re-extraction is necessary to isolate the majority of the poly A⁺ RNA from the sample. More than two rounds are not recommended as the third and fourth round of extraction tend to have lower yields and poorer 260/280 ratios. Follow steps 5.3.1 to 5.3.4 to prepare beads for each round of re-extraction.

NOTE: The beads may be used for up to 3 rounds of extraction (of the same sample). New beads should be used for different samples to prevent cross contamination.

NOTE: Subsequent elutions may be combined with the first-round elution or kept separately.

5.3.1 Place tube into MPC for 1 minute.

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5.3.2 Remove and discard buffer without disturbing pellet.

5.3.3 Remove tube from MPC and resuspend beads in the buffer-sample solution from step 5.2.7.

5.3.4 Proceed with the protocol from steps 5.2.4 until 5.2.14 for each round of re-extraction.

5.4 Clean-Up

5.4.1 Discard buffer-sample solution and beads.

5.4.2 Use spectrophotometer to measure concentration (260nm) and ratio (260nm vs. 280nm) of poly A⁺ RNA. Good quality poly A⁺ RNA have ratios between 1.9 and 2.1. Typical yields range between 0.5% and 3% of total RNA yield.

5.4.3 Store poly A⁺ RNA at -80° C as soon as possible.