NEXTFLEX®
Poly(A) Beads 2.0

(Compatible with Illumina® and Element® platforms)

KIT CONTAINS: 8, 48, or 96 RXNS | 10 ng -5 µg Total RNA

USER MANUAL FOR :
#NOVA-512991
#NOVA-512992
#NOVA-512993

NEXTFLEX® Poly(A) Beads 2.0

This product is for research use only.

Not for use in diagnostic procedures.

GENERAL INFORMATION

Product Overview

The NEXTFLEX® Poly(A) Beads 2.0 are designed to isolate pure, intact messenger RNA (mRNA). The procedure includes two bead-based steps to ensure maximum removal of ribosomal RNA (rRNA) and non-messenger RNA contaminants. Magnetic based separation is used to retain poly(A) mRNA while removing all other transcripts. Beads are subsequently washed and mRNA is eluted. mRNA comprises approximately 1-5% of total RNA. For example, the maximum expected yield from 5 µg is 250 ng poly(A) RNA. The following bead and total RNA ratios are suggested for optimal binding capacity and yield NEXTFLEX® Poly(A) Beads 2.0 are specifically designed and tested for RNA-seq applications.

The NEXTFLEX® Poly(A) Beads 2.0 has been functionally validated using the NEXTFLEX® Rapid Directional RNA-Seq Kit 2.0 and NEXTFLEX® RNA-Seq 2.0 UDI Barcodes. For additional product details, please contact us at https://www.revvity.com/contact-us/technical-support.

Kit Contents, Storage & Shelf Life

This kit contains enough material to extract mRNA from 8, 48, or 96 total RNA samples of 10 ng - 5 µg total RNA for use with RNA-seq applications. The shelf life of all reagents is 12 months when stored properly. The NEXTFLEX® Poly(A) Elution Buffer 2.0, NEXTFLEX® Poly(A) Binding Buffer 2.0, NEXTFLEX® Poly(A) Washing Buffer 2.0, and NEXTFLEX® Poly(A) Beads 2.0 should be stored at 4°C.

Kit Contents	Cap Color	Amount (8/48/96 rxns)	Storage Temp.
NEXTFLEX® Poly(A) Beads 2.0	YELLOW CAP	160/960/(2) 960 µL	4°C
NEXTFLEX® Poly (A) Elution Buffer 2.0	YELLOW CAP	1/6*/12* mL	4°C
NEXTFLEX® Poly(A) Binding Buffer 2.0	YELLOW CAP	(2) 1/15*/30* mL	4°C
NEXTFLEX® Poly(A) Washing Buffer 2.0	CLEAR CAP BOTTLE	4 /24/48 mL	4°C

** In White Cap Bottle*

Required Materials Not Provided

- 10 ng - 5 µg total RNA
- Nuclease-free water
- Thermal Cycler
- 96 well PCR Plate Non-skirted (Phenix Research™, # MPS-499) or similar
- Adhesive PCR Plate Seal (BioRad®, # MSB1001)
- Magnetic Stand (ThermoFisher® Scientific, # AM10027)
- RNase-free barrier pipette tips

- 2, 10, 20, 200 and 1000 µL pipettes
- RNase-free pipette tips
- Nuclease-free 1.5 mL microcentrifuge tubes
- Microcentrifuge
- Vortex

Warnings & Precautions

- We strongly recommend that you read the following warnings and precautions. Periodically, optimizations and revisions are made to the components and manual. Therefore, it is important to follow the protocol included with the kit. If you need further assistance, you may contact your local distributor, or contact us at https://www.revvity.com/contact-us/technical-support and choose the "Next Gen Sequencing" category.
- Do not use the kit past the expiration date.
- Try to maintain a laboratory temperature of 20°–25°C (68°–77°F).
- RNA sample quality may vary between preparations. High quality RNA should have a 28S band that is twice as intense as the 18S band of ribosomal RNA.
- Vortex beads until they are a uniform suspension.
- Keep beads in liquid suspension during storage and handling. Never freeze the beads.
- Ensure beads pellet on magnet before removing clear supernatant. Completely remove all washing buffer before eluting mRNA.
- We recommend examining total RNA integrity using a LabChip® or equivalent.
- High quality total RNA preparations should have an RNA Integrity Number (RIN) or RNA Quality Score (RQS) greater than or equal to 7

Starting Materials

The NEXTFLEX® Poly(A) Beads 2.0 have been optimized and validated using 10 ng – 5 µg of total RNA for RNA-seq applications. High quality total RNA preparations should have an RNA Integrity Number (RIN) ≥7. Alternatively, total RNA may be run on a 1–2% agarose gel and integrity examined by staining with ethidium bromide. High quality RNA should have a 28S band that is twice as intense as the 18S band of ribosomal RNA.

Revision History

Version	Date	Description
V19.06	June 2019	Early Market Release
V19.10	October 2019	Product Launch
V21.01	January 2021	Manual Update
V22.01	January 2022	Manual Update
V23.10	October 2023	Rebrand

SAMPLE PREP PROTOCOL

Step A: NEXTFLEX® Poly(A) Bead Preparation Procedure

MATERIALS

YELLOW CAP - NEXTFLEX® Poly (A) Beads 2.0

YELLOW CAP or WHITE CAP BOTTLE - NEXTFLEX® Poly(A) Binding Buffer 2.0

User Supplied

- Total RNA (10 ng – 5 µg) in nuclease-free water
- 96-well PCR plate
- 96-well PCR plate magnetic stand
- Nuclease-free water as needed

This procedure takes approximately 10 minutes to complete and should be carried out before starting mRNA purification.

1. Thoroughly resuspend NEXTFLEX® Poly(A) Beads 2.0 by vortexing. Ensure no beads remain settled at the bottom of the bottle or tube.
2. Transfer 100 µL of NEXTFLEX® Poly(A) Binding Buffer 2.0 to fresh wells of a new 96-well PCR plate. Each sample requires its own well.
3. Add 20 µL of NEXTFLEX® Poly(A) Bead 2.0 to each well and mix until homogenous. Each well must contain 100 µL of NEXTFLEX® Poly(A) Binding Buffer 2.0 and 20 µL of NEXTFLEX® Poly(A) Beads 2.0.
4. Place the 96-well PCR plate on the magnet for 30 seconds or until solution becomes clear.
5. Discard the clear supernatant while the 96-well PCR plate remains on the magnet.
6. Remove 96-well PCR plate from magnet.
7. Resuspend pellet in 50 µL of NEXTFLEX® Poly(A) Binding Buffer 2.0. Proceed to Step B: Purification of mRNA from total RNA.

Step B: Purification of mRNA from total RNA

MATERIALS

YELLOW CAP - NEXTFLEX® Poly (A) Beads 2.0

YELLOW CAP or WHITE CAP BOTTLE - NEXTFLEX® Poly (A) Binding Buffer 2.0

WHITE CAP BOTTLE - NEXTFLEX® Poly (A) Wash Buffer 2.0

User Supplied

- Total RNA (10 ng – 5 µg) in nuclease-free water
- Nuclease-free water as needed
- Thermal cycler
- 96-well PCR plate
- 96-well PCR plate magnetic stand

First Bead Binding

Bring volume of total RNA sample to 50 µL using nuclease-free water.

1. Add 50 µL total RNA to the prepared beads from Step A. Mix thoroughly until homogenized.
2. Each well must contain 50 µL of the total RNA sample and 50 µL of the prepared beads.
3. Apply adhesive PCR plate seal and place in thermal cycler that is programmed as follows:

5 min	65°C
HOLD	4°C

4. Remove the 96-well PCR plate from the thermal cycler.
5. Mix thoroughly until homogenized.
6. Incubate at room temperature for 5 minutes.

First Bead Wash

1. Place the 96-well PCR plate on the magnetic stand at room temperature for 30 seconds or until the supernatant appears completely clear.
2. Remove and discard clear supernatant taking care not to disturb beads.
3. Remove the 96-well PCR plate from the magnetic stand.
4. Resuspend pellet with 200 µL of NEXTFLEX® Poly(A) Washing Buffer 2.0. Mix thoroughly until homogenized.
5. Place the 96-well PCR plate on the magnetic stand for 30 seconds or until supernatant appears completely clear.
6. Remove and discard clear supernatant taking care not to disturb beads.
7. Remove the 96-well PCR plate from the magnetic stand.
8. Resuspend pellet with 50 µL of NEXTFLEX® Poly(A) Elution Buffer 2.0.

Second Bead Binding

1. Apply adhesive PCR plate seal and place in thermal cycler that is programed as follows:

5 min	70°C
HOLD	25°C

2. Add 50 µL of NEXTFLEX® Poly(A) Binding Buffer 2.0 to each sample. Mix thoroughly until homogenized. Do not magnetize.
3. Incubate at room temperature for 5 minutes.

Second Bead Wash

1. Place the 96-well PCR plate on the magnetic stand at room temperature for 30 seconds or until the supernatant appears completely clear.
2. Remove and discard clear supernatant taking care not to disturb beads.
3. Remove the 96-well PCR plate from the magnetic stand.
4. Resuspend pellet with 200 µL of NEXTFLEX® Poly(A) Washing Buffer 2.0. Mix thoroughly until homogenized.
5. Place the 96-well PCR plate on the magnetic stand for 30 seconds or until supernatant appears completely clear.
6. Remove and discard clear supernatant taking care not to disturb beads.
7. Remove 96-well PCR plate from the magnetic stand.

Final Elution

1. Resuspend pellet with 17 µL of NEXTFLEX® Poly(A) Elution Buffer 2.0.
2. Place the 96-well PCR plate in a thermal cycler that is programmed as follows:

5 min	70°C

3. Transfer the hot 96-well PCR to a magnet. Do not allow the 96-well PCR plate to cool to room temperature.
4. Transfer 14 µL of clear sample to a new well. mRNA is now ready for RNA-seq based applications.
5. For total RNA inputs greater than or equal to 1 µg, fluorometric analysis using a Qubit fluorometer and electropherogram analysis using a Labchip® instrument is recommended.

Representative Poly(A) Enrichment Profile

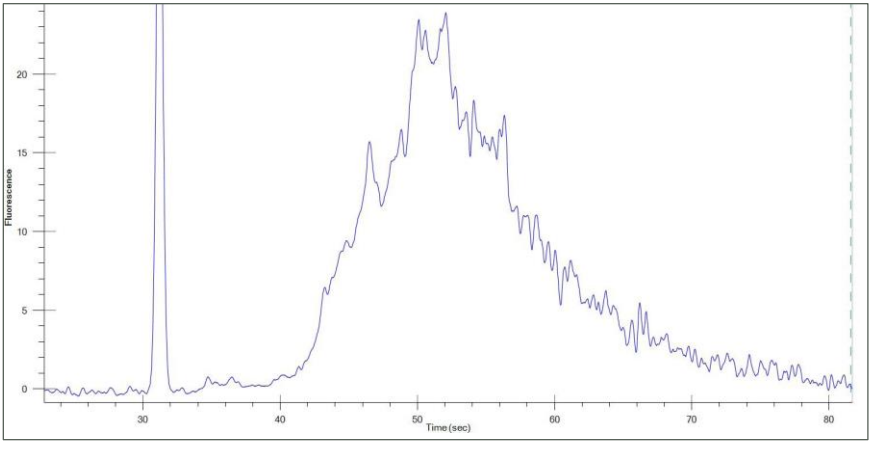

Figure 1: Example of 1 µg of Universal Human Reference (Agilent® #740000) after Poly A enrichment.

3 µL Poly(A) enriched RNA was run on the LabChip® GXII Touch™ HT instrument using the RNA Pico Assay Reagent Kit (# CLS960012) and a DNA/RNA/Charge Variant Assay LabChip (# 760435).

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