Poly(A) Tail-Length Assay Kit

Product Number 76450
5 G/I Tailing, 20 RT, and 400 PCR reactions

STORAGE

Store at -20°C.

Warning: For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

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CONTENTS

COMPONENTS

All reagents have been extensively tested and carefully prepared to meet USB® standards. It is recommened that the reagents be used as directed in order to achieve the best possible results.

This kit contains reagents sufficient for 5 G/I tailing, 20 reverse transcription, and 400 PCR reactions. In addition, this kit includes HeLa total RNA and control human actin PCR forward primer that can be used to verify components and protocol.

The following components are included with each kit:

5X Tail Buffer Mix	25 µl
10X Tail Enzyme Mix	12 µl
10X Tail Stop Solution	15 µl
5X RT Buffer Mix (includes RT primer)	100 µl
10X RT Enzyme Mix	50 µl
5X PCR Buffer Mix	2 x 1.2 ml
Universal PCR Reverse Primer, 10µM	410 µl
HotStart-IT® Taq DNA Polymerase, 1.25 units/µl	2 x 250 units
Control, human actin PCR Forward Primer, 10µM	8 µl
Control, HeLa Total RNA, 100 ng/µl	10 µl
$MgCl_2$, 25mM	1 ml
Water, Nuclease-Free	8 x 1 ml

The enclosed reagents should be stored at -15°C to -30°C (NOT in a frost-free freezer). HeLa total RNA should be stored at -80°C. After thawing for use, keep reagents on ice.

QUALITY CONTROL

The Poly(A) Tail-Length Assay Kit is a Tested User Friendly™ product assuring reliable results. This kit is functionally tested for *actin* and *k-ras* poly(A) tail-length detection from HeLa total RNA following the protocol in the manual. All components were tested for contaminating ssDNA and dsDNA endonucleases, ssDNA and dsDNA exonucleases, and ribonucleases. Properly handled and stored components are guaranteed for optimal performance for at least 6 months from the date received.

SAFETY WARNINGS AND PRECAUTIONS

Warning: For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

Caution: All chemicals should be considered as potentially hazardous. We, therefore, recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing, such as lab coat, safety glasses, and gloves. Care should be taken to avoid contact with skin and eyes. In the case of contact with skin or eyes, wash immediately with water. See MSDS (Material Safety Data Sheet) for specific advice.

INTRODUCTION

The poly-adenylated tail (poly(A) tail) on nearly all eukaroyotic mRNAs plays a number of important roles in mRNA metabolism including enhancing translation, mRNA stability and transport from the nucleus[1,2]. Studies in several model organisms have shown regulated deadenylation is rate limiting for mRNA degradation. Importantly, deadenylation is now recognized as a mechanism of miRNA mediated gene regulation[3,4]. Thus, identifying changes in poly(A) tail-length can yield insights into mRNA regulation and subsequent physiological impact.

The Poly(A) Tail-Length Assay Kit uses four key steps to enable poly(A) tail-length determination. In Step 1, poly(A) polymerase adds a limited number of guanosine and inosine residues to the 3'-ends of poly(A)-containing RNAs[5,6]. In Step 2, the tailed-RNAs are converted to cDNA through reverse transcription using the newly added G/I tails as the priming sites. In Step 3, PCR amplification products are generated using two primer sets. A gene-specific forward and reverse primer set designed upstream of the polyadenylation site (*e.g.* the 3'-UTR) is produced as a control for the gene-of-interest. The second set of primers uses the gene-specific forward primer and the universal reverse primer provided with the kit to generate a product that includes the poly(A) tails of the gene-of-interest. Finally, in Step 4, the PCR products are separated on an agarose or polyacrylamide gel. The poly(A) tail-lengths of the gene-of-interest are the sizes of poly(A) PCR-amplified products minus the calculated length of the gene-specific forward primer to the putative polyadenylation start site.

ASSAY PROCEDURE OVERVIEW

The Poly(A) Tail-Length Assay Kit is designed for G/I tailing up to five samples of total RNA. All necessary components are provided to perform 4 reverse transcription and 80 PCR reactions on each of the five tail-extended samples. Reaction products are then assessed by gel electrophoresis.

The protocol includes the following steps:

- Step 1: G/I Tailing (60 min incubation)
- Step 2: Reverse Transcription (70 min incubation)
- Step 3: PCR Amplification (30-60 min incubation)
- Step 4: Detection

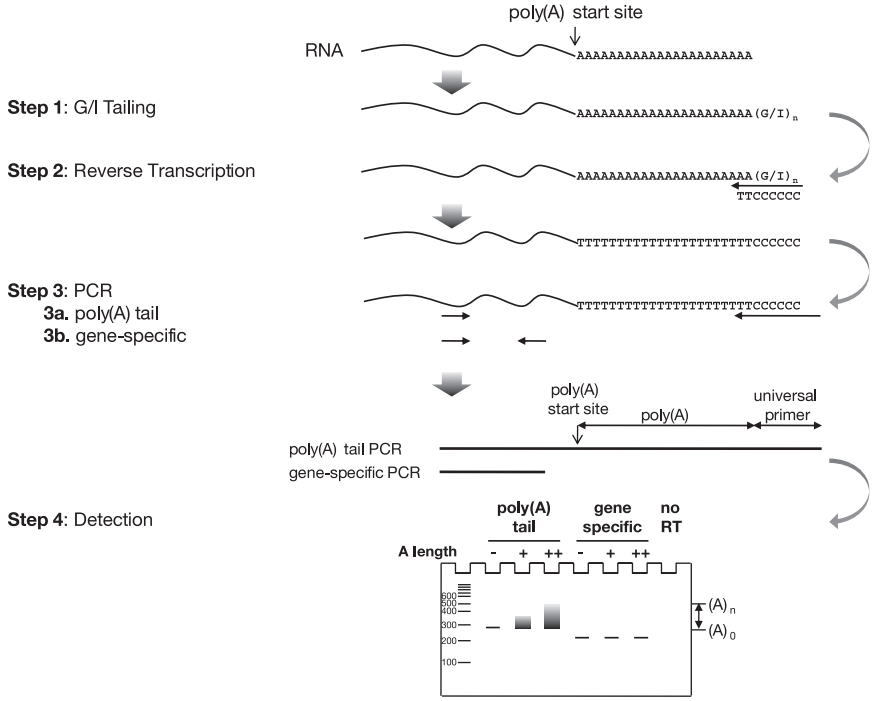


Figure 1. Poly(A) Tail-Length Assay Procedure.

MATERIALS NOT SUPPLIED

The following materials are required for use with the kit:

- 100 ng to 2 µg of total RNA (see Starting Materials and Related Product sections for advice and sample preparation kits)
- Specific PCR forward and reverse primers designed for the gene-of-interest (see Supplementary Information for design guidelines)
- Microcentrifuge
- Thermal cycler
- Adjustable precision pipettes
- RNase-free filter pipette tips and Nuclease-free tubes
- Appropriate PCR plates/tubes for instrument
- Disposable gloves
- Gel electrophoresis
  - Molecular weight marker (USB PN 76712 or 76710)
  - DNA loading buffer (USB PN 76715 or 76720)
  - 2-2.5% agarose (USB PN 32802) gel and TAE buffer (USB PN 75904 or 74015)
  - 4-6% non-denaturing polyacrylamide (USB PN 75848) and TBE buffer (USB PN 75891)
  - UV transilluminator or fluorescence image scanner

PROTOCOL

Reagent and Sample Handling

Thaw reagents on ice, mix thoroughly before use and immediately return unused materials to -20°C. When preparing working reagents, measure components accurately, mix thoroughly, spin briefly and keep on ice. Assemble reactions on ice or at the indicated temperature throughout the procedure.

When working with RNA, wear gloves at all times while handling reagents, materials and equipment to prevent RNase contamination from hands. Clean pipettes and work areas with RNaseAway™ or RNaseZap® to reduce the risk of RNase contamination. Use RNase-free plastic ware and RNase-free buffers and reagents.

Starting Materials

A typical assay reaction uses 0.1 – 2 µg of total RNA. The amount of total RNA required per assay depends on the target abundance in the sample. It is important to use RNA that is completely free of contaminating genomic DNA. It is generally unnecessary to treat the RNA with DNase I to remove any genomic DNA contamination. However, certain RNA preparations may yield non-specific amplification products that can be removed by treating the isolated RNA with rDNase I (PN 78311). Samples treated with DNase I should be extracted with phenol-chloroform or purified with a column-based procedure.

Assay Controls

Prepare an “Assay Positive Control” by using the supplied HeLa Total RNA and human actin PCR Forward Primer. This control will be used to assess assay components and procedures.

Prepare a “No RT Negative Control” to assess non-specific amplification by substituting the 10X RT Enzyme Mix with Nuclease-Free Water.

Prepare a “Specific Primer Control” to assess specificity of the gene-specific PCR forward primer by substituting the Universal PCR Reverse Primer with the gene-specific PCR reverse primer (not supplied).

The following table summarizes the recommended reactions that should be performed.

Step	G/I Tailing			Reverse Transcription		PCR Amplification			
						Tail PCR Primers		Specific PCR Primers	
	Input	Enzyme	Buffer	Enzyme	Buffer	Forward	Reverse	Forward	Reverse
Assay Positive Control	✓HeLa RNA	✓	✓	✓	✓	✓actin	✓Universal	n/a	n/a
Sample									
No RT Negative Control	test RNA	✓	✓	water	✓	S	✓Universal	n/a	n/a
Specific Primer Control	test RNA	✓	✓	✓	✓	n/a	n/a	S	S
Poly(A) Test	test RNA	✓	✓	✓	✓	S	✓Universal	n/a	n/a

✓ indicates use of supplied components.
S indicates gene-specific.

Thermal Cycler Programs

During the Poly(A) Tail-Length Assay, the samples are placed in a thermal cycler three times. Therefore, we recommend programming your thermal cycler(s) with the following programs prior to sample processing.

Programs

1. G/I Tailing: 37°C for 60 min
2. Reverse Transcription: 44°C for 60 min; 92°C for 10 min; and 4°C hold
3. PCR Amplification:

Two-Step PCR, **Recommended**	Three-Step PCR
94°C for 2 min 30-35 cycles of: 94°C for 10 sec 60°C for 30-60 sec 72°C for 5 min 4°C hold	94°C for 2 min 30-35 cycles of: 94°C for 10 sec 58°C for 30 sec 72°C for 30 sec 72°C for 5 min 4°C hold

Note: Certain targets may exhibit sub-optimal amplification with the Two-Step PCR protocol. The Three-Step PCR protocol should be used in cases where weak PCR amplification is observed.

PROTOCOL

Step 1: G/I Tailing

Use the following protocol to add poly(G/I) tails to a total RNA sample. For the positive control, substitute the provided HeLa total RNA for an experimental sample. This standard protocol applies to a single 20 µl G/I Tailing reaction.

1. Thaw frozen reagents on ice and mix thoroughly by vortexing. Enzyme mixes should be gently flicked to mix. Centrifuge briefly.
2. Add the following reagents in Table 1 to a nuclease-free tube. Mix gently by pipetting up and down and then centrifuge the tube briefly to collect the contents. Keep samples on ice.

Table 1. G/I Tailing Mix

Reagent	Per reaction
Total RNA sample, 1 µg (0.1 – 2 µg)	up to 14 µl
5X Tail Buffer Mix	4 µl
10X Tail Enzyme Mix	2 µl
Water, Nuclease-Free	to 20 µl

3. Incubate at 37°C for 60 min
4. Add 2 µl 10X Tail Stop Solution and mix well.
5. Proceed to Step 2: Reverse Transcription. *Alternatively, tailed RNA samples can be stored at -20°C until ready to proceed to Step 2.*

Step 2: Reverse Transcription

Use the following protocol to reverse transcribe the poly(G/I) tailed RNA. This standard protocol applies to a single 20 µl reverse transcription reaction. Master mixes for multiple reactions can be made by increasing the volumes of reaction components proportionally.

1. Thaw frozen reagents on ice and mix thoroughly by vortexing. Enzyme mixes should be gently flicked to mix. Centrifuge briefly.
2. Add the following reagents in Table 2 to a nuclease-free tube. Mix gently and briefly spin down the tube contents. Keep on ice.

Table 2. RT Mix

Reagent	RT +	RT - (control)
G/I Tailed RNA Sample	5 µl	5 µl
5X RT Buffer Mix	4 µl	4 µl
10X RT Enzyme Mix	2 µl	-
Water, Nuclease-Free	9 µl	11 µl

Note: Each kit supports 20 x 20 µl reactions.

3. Incubate at 44°C for 60 min; 92°C for 10 min; and at 4°C hold.
4. Proceed to Step 3: PCR Amplification. *Alternatively, cDNA samples can be stored at -20°C until ready to proceed to Step 3.*

Step 3: PCR Amplification

Use the following protocol to PCR amplify the poly(G/I) tailed cDNA. This standard protocol applies to a single 25 µl PCR reaction. Master mixes for multiple reactions can be made by increasing the volumes of reaction components proportionally.

1. Dilute each RT sample by adding 20 µl Nuclease-Free Water (40 µl final volume).
2. Thaw frozen reagents on ice and mix thoroughly by vortexing. Mix HotStart-IT® Taq DNA Polymerase by gently flicking. Centrifuge briefly.
3. Add the following reagents in Table 3 to a nuclease-free tube. Mix gently and briefly spin down the tube contents. Keep on ice.

Table 3. PCR Mix

Reagent	RT + Tail PCR	RT - Tail PCR	RT + Specific PCR	RT - Specific PCR
Diluted RT sample	up to 5 µl	up to 5 µl	up to 5 µl	up to 5 µl
5X PCR Buffer Mix	5 µl	5 µl	5 µl	5 µl
10 µM Gene-Specific PCR Forward Primer	1 µl	1 µl	1 µl	1 µl
10 µM Universal PCR Reverse Primer	1 µl	1 µl	-	-
10 µM Gene-Specific PCR Reverse Primer	-	-	1 µl	1 µl
25mM $MgCl_2$*	optional	optional	optional	optional
1.25 units/µl HotStart-IT® Taq DNA Polymerase	1 µl	1 µl	1 µl	1 µl
Water, Nuclease-Free	to 25 µl	to 25 µl	to 25 µl	to 25 µl

*Additional $MgCl_2$ may be required to increase amplification efficiency of certain targets and is provided in this kit.

4. Proceed to Step 4: Detection. *Alternatively, PCR products can be stored at -20°C until ready to proceed to Step 4.*

Step 4: Detection

The size of PCR products can be assessed by running an aliquot of the reaction on an agarose or polyacrylamide gel. To start, we recommend loading one half of each PCR reaction (12.5 µl) per lane on a 2.5% agarose TAE gel. For increased resolution, load one half of each PCR reaction (12.5 µl) per lane on a 5% non-denaturing polyacrylamide TBE gel. Stain gels with ethidium bromide or SYBR® Gold and visualize with a standard ultraviolet transilluminator or fluorescence image scanner.

See the Supplementary Information Section for guidelines on gel electrophoresis and data analysis.

SUPPLEMENTARY INFORMATION

Data Analysis

The Poly(A) Tail-Length Assay Kit determines the length distribution of mRNA poly(A) tails. PCR products of mRNAs with short tails will yield discrete bands, whereas mRNAs with long tails will yield a smear on the gel (Fig. 1). PCR amplification with the gene-specific forward primer and Universal reverse primer amplifies the sequence upstream of the polyadenylation start site site (*e.g.* the 3'-UTR) to the end of the poly(A) tails. The poly(A) tail-lengths of the gene-of-interest are the sizes of poly(A) PCR-amplified products minus the calculated length of the gene-specific forward primer to the putative polyadenylation start site (Fig. 2). PCR with the gene-specific forward and reverse primers should amplify only the upstream sequence of the expected size to validate the specificity of the gene-specific forward primer. The “No RT Negative Control” reaction should have no signal. Examples of results are shown in Figs. 3 and 4.

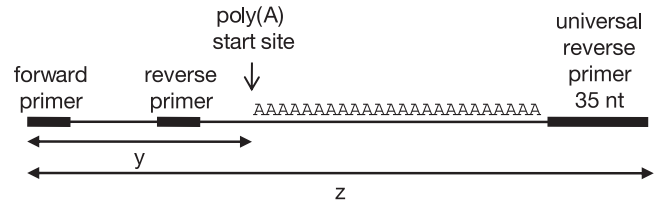


Figure 2. Example of poly(A) tail-length determination. A-tail length is (z-y-35) where z can vary based on gel results.

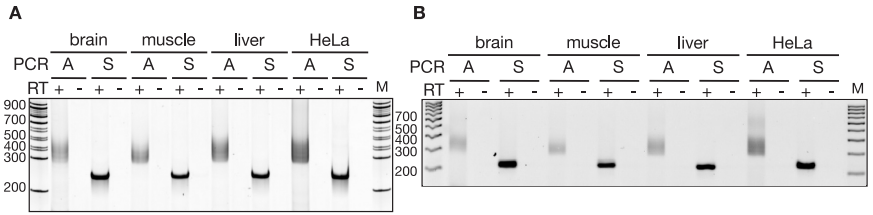


Figure 3. Comparison of human actin poly(A) tail-lengths in brain, muscle, liver and HeLa cell. One microgram total RNA and 4 µl of diluted RT samples were used in G/I Tailing and PCR reactions, respectively. The recommended two-step PCR program was used. One half of each PCR reaction (12.5 µl) was analyzed on 6% non-denaturing polyacrylamide-TBE gel stained with SYBR® Gold **(A)**, and 2.5% agarose-TAE gel stained with ethidium bromide **(B)**. RT (+); No RT Negative Control (-); poly(A) tail PCR (A); gene-specific PCR (S); and 100 bp DNA Ladder (USB PN 76712) (M).

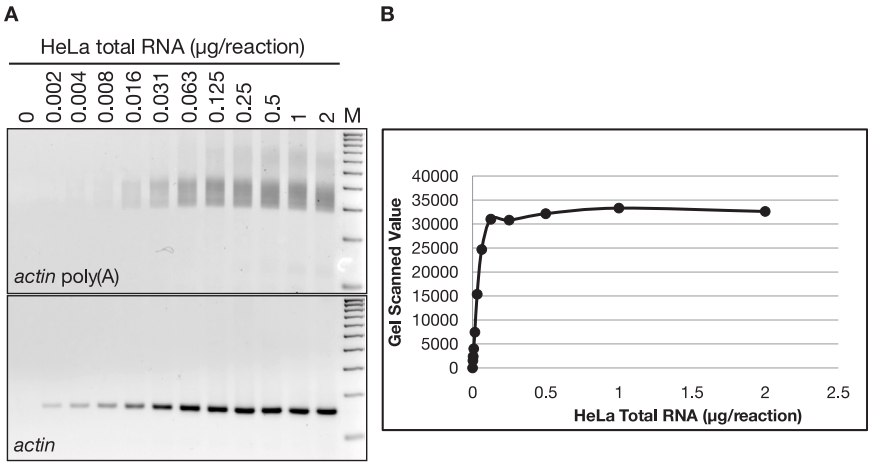


Figure 4. Detection sensitivity of the USB Poly(A) Tail-Length Assay. Actin poly(A) tail-length was determined from a two-fold serial dilution HeLa total RNA. Samples were processed as described in Fig. 3B (**A**). The top image was quantified by densitometry (**B**).

PCR Primer Design

Universal reverse primer: The Universal PCR Reverse Primer supplied with each kit is used as the reverse primer in poly(A) tail-length detection PCR reactions. It is supplied at 10µM and used at a final concentration of 400nM.

Gene-specific forward and reverse primers: These are the primers that are user-defined for the gene-of-interest. They should be diluted to 10µM in TE Buffer (PN 75893) and used at a final concentration of 400nM. The forward primer is used with the universal reverse primer to generate the poly(A) tail-length PCR products and the gene-specific forward and reverse primers are used together to verify the specificity of the forward primer and the presence of the target within the RNA sample.

The gene-specific PCR primers should be located within 50-300 nucleotides upstream of the poly(A) start site to allow proper resolution of PCR products by gel electrophoresis. If possible, the gene-specific reverse primer should be located immediately upstream of the poly(A) start site for straightforward calculation of the poly(A) tail-lengths. We recommend using computer programs designed to select appropriate primers in a given sequence. Several public primer databases are available on the internet. Some examples of databases include:

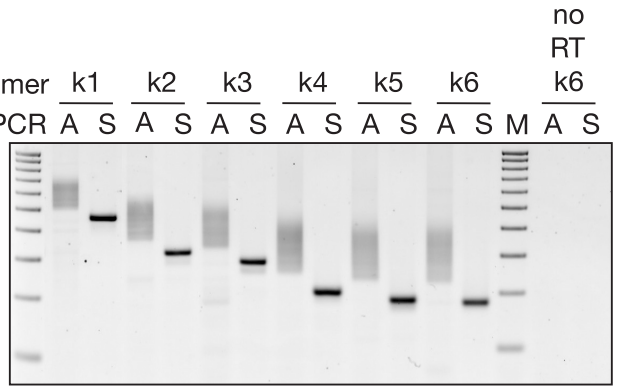
NCBI, http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome
Primer3, http://frodo.wi.mit.edu
IDT, http://www.idtdna.com/Scitools/Applications/Primerquest

In general, follow these guidelines for best results:

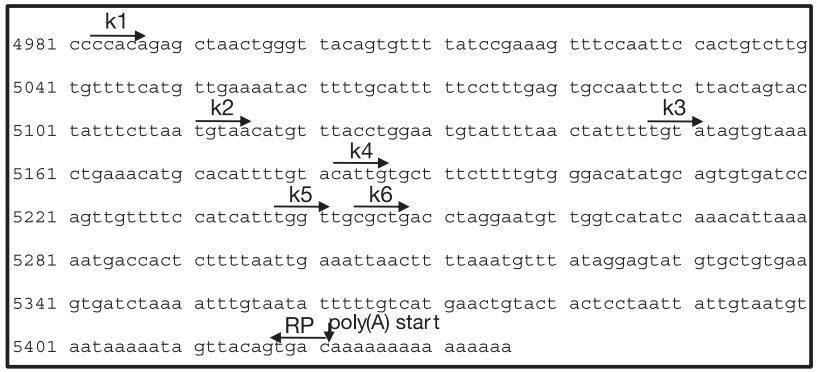
- Primers should range in length from 19 to 30 nucleotides,
- G+C content in the range of 30 to 50%,
- T_m values ranging from 55-60°C,
- Analyze for cross-reactivity in the organism's database.

Due to the AT-rich content in 3' UTR sequences, it may be difficult in some cases to design a primer that fits these specifications. We have also tested that primers with T_m below 55°C and have found that these can work in this assay as long as the gene-specific forward primer has been validated for specific priming and amplification of the gene-of-interest. In general, two specific forward primers and one specific reverse primer should be designed per gene-of-interest for best possible results. An example of using different specific forward primer designs for poly(A) tail-length determination is shown in Fig. 5.

A



B



C

k-ras primer	5' → 3' Sequence	T_m (°C)	GC (%)	Length (nt)
k1	CCACAGAGCTAACTGGGTTACAGT	58.4	50	24
k2	TGTAACATGTTTACCTGGAATGT	52.3	35	23
k3	TGTATAGTGTAAACTGAAACATGCAC	53.6	35	26
k4	CATTGTGCTTTCTTTTGTGGGACA	56.5	42	24
k5	TGGTTGCGCTGACCTAGGAATGTT	60.8	50	24
k6	CGCTGACCTAGGAATGTTGG	55.6	55	20
k-RP	GTCACTGTAACTATTTTTATTAC	45.2	26	23

Figure 5. Different gene-specific forward primer designs for poly(A) tail-length determination of *k-ras* from HeLa total RNA. Primer location on *k-ras* transcript (**B**) and primer information are shown (**C**). Samples were processed as described in Fig. 3B (**A**). No RT Negative Control (RT -); poly(A) tail PCR (A); gene-specific PCR (S); and 100 bp DNA Ladder (USB PN 76712) (M).

Analysis by Gel Electrophoresis

Preparing and running agarose gels

1. Choose a horizontal gel electrophoresis apparatus with a capacity of ≥15 µl per well.
2. Prepare 2.5% agarose TAE gel by mixing 2.5 gm agarose (PN 32802) per 100 ml 1X TAE Buffer (*e.g.* PN 75904 or 74015, diluted to 1X with distilled water).
3. Heat to boil the agarose until completely dissolved.
4. Cool to ~65°C, then add ethidium bromide to 1 µg/ml (or 1 drop of ethidium bromide, PN 75816, per 100 ml).
5. Pour the gel solution into the gel tray with comb to form wells and let set completely.
6. Prepare sample by adding loading buffer to 1X (*e.g.* 4 µl of 6X Loading Buffer, PN 76715 or PN 76720).
7. Mix and quick spin to collect tube contents at the bottom of the tubes.
8. Load 14 µl of the dye-PCR mix sample per lane. For the first and the last lane, load DNA marker (*e.g.* 3 µl of 100 bp DNA Ladder, PN 76712).
9. Run in 1X TAE Buffer (*e.g.* PN 75904, diluted to 1X with distilled water) at 150 volts for 40-60 min.
10. Visualize and document with a standard ultraviolet transilluminator or fluorescence image scanner.

Preparing and running polyacrylamide gels

1. Choose a vertical gel electrophoresis apparatus with a capacity of ≥15 µl per well. Follow the manufacturer's instructions for the details of assembling gel apparatus.
2. One 10 cm x 15 cm x 1 mm gel requires 15 ml of gel solution. Prepare 5% polyacrylamide TBE gel by mixing the following:

For 15 ml	
5X TBE (PN 75891)	3 ml
40% acrylamide solution (19:1 acrylamide:bis-acrylamide, PN 75848)	1.9 ml
water to 15 ml	10.1 ml
Add the following reagents immediately before pouring the gel: 10% ammonium persulfate (PN 76322) in water TEMED (PN 76320)	 120 µl 16 µl

3. Pour the gel solution into the gel cassette and place comb to form wells and let polymerize completely at room temperature for at least 30 min.
4. Prepare sample by adding loading buffer to 1X (*e.g.* 4 µl of 6X Loading Buffer, PN 76715 PN 76720).
5. Mix and quick spin to collect tube contents at the bottom of the tubes.
6. Load 14 µl of the dye-PCR mix sample per lane. For the first and the last lane, load DNA marker (*e.g.* 3 µl of DNA Ladder, 100 bp, USB PN 76712).
7. Run in 1X TBE Buffer (*e.g.* PN 75891, diluted to 1X with distilled water) at ~7 watt, constant power or ~25 mAmp, constant current for 30-60 min.
8. Stain with SYBR® Gold Nucleic Acid Gel Stain (Life Technologies) according to the manufacturer's instructions.
9. Visualize and document with a standard ultraviolet transilluminator or fluorescence image scanner.

TROUBLESHOOTING

Problem	Possible causes and solutions
Weak or no signal	1. Poor RNA sample quality – Check RNA integrity by gel electrophoresis or bioanalyzer. 2. Low abundant RNA target – Increase the amount of total RNA to 2 µg per G/I Tailing reaction. – Use poly(A)-enriched RNA. Up to 0.5 µg poly(A) RNA sample per reaction can be used. – Increase the sample volume for gel analysis. 3. Sub-optimal PCR condition – Increase the amount of diluted RT to 5 µl per PCR reaction. – Optimize $MgCl_2$ for the PCR reaction. – Try different PCR forward primer. – Increase the number of PCR cycles. – Decrease PCR annealing temperature. – Increase PCR extension time. – Try the Three-Step PCR protocol. – Use the supplied PCR reagents. These components have been optimized for use with this assay.
Non-specific signal	1. Poor RNA sample quality – This may indicate the presence of contaminating genomic DNA in the RNA sample. Treat the RNA sample with DNase I and remove the DNase I by phenol-chloroform extraction or a column-based purification. 2. Isoform detection – Check if the gene-of-interest has different isoforms and the unexpected signals correspond to the presence of alternatively spliced transcripts. – Design new specific forward primers that allow isoform discrimination.

Problem	Possible causes and solutions
	3. Sub-optimal PCR condition – Use the recommended Two-Step PCR protocol. – Decrease the number of PCR cycles. – Design new specific primers. – Use the supplied PCR reagents. These components have been optimized for use with this assay.
	4. DNA contamination during sample processing – Use filter-barrier tips for assay set-up. – Replace all reagents for PCR.

If problems persist please contact Technical Support for assistance at (800) 321-9322 or USBtechsupport@affymetrix.com. For technical support outside the U.S., please visit our website for up-to-date contact information on the USB product distributor within your area.

REFERENCES

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2. Andersen K.R., Jensen T.H., and Brodersen D.E. (2008) *Biochim Biophys Acta*. **1779**, 532-537.
3. Wu L., Fan J., and Belasco J.G. (2006) *Proc Natl Acad Sci USA.* **103**, 4034-4039.
4. Eulalio A., Huntzinger E., Nishihara T., Rehwinkel J., Fauser M., and Izaurralde E. (2009) *RNA* **15**, 21-32.
5. Martin G., and Keller W. (1998) *RNA* **4**, 226-230.
6. Kusov Y.Y., Shatirishvili G., Dzagurov G., and Gauss-Müller V. (2001) *Nucleic Acids Res.* **29**, E57-7.

RELATED PRODUCTS

Product	Application	Pack size	Product number
PrepEase® RNA Clean-Up Kit	Clean-up of RNA	10 preps 50 preps 250 preps	78875 78876 78877
Agarose LE	Gel electrophoresis	25 gm 100 gm 250 gm 500 gm 1 kg	32802

Product	Application	Pack size	Product number
Ammonium Persulfate	Gel electrophoresis	100 gm	76322
DNA Ladder, 100 bp	Gel electrophoresis	500 µl	76712
DNA Loading Buffer, 6X (included with 76710 and 76712)	Gel electrophoresis	1 ml 5 ml	76715
6X DNA Loading Buffer (BXF)	Gel electrophoresis	1 ml 5 ml	76720
Ethidium Bromide Drops	Gel electrophoresis	5 ml	75816
PCR Markers, 50-2,000 bp	Gel electrophoresis	250 µl	76710
RapidGel, 40% Liquid Acrylamide Stock Solution	Gel electrophoresis	500 ml	75848
TAE Buffer, 10X Solution	Gel electrophoresis	1 L 5 L	75904
TAE Buffer, 50X Solution	Gel electrophoresis	100 ml	74015
TBE Buffer, 5X Solution	Gel electrophoresis	1 L 5 L	75891
TEMED	Gel electrophoresis	100 ml	76320
PrepEase® mRNA Mini Spin Kit	Isolation of mRNA	12 preps	78878
PrepEase® RNA Spin Kit	Isolation of RNA	10 preps 50 preps 250 preps	78765 78766 78767
PrepEase® RNA SVE Spin Kit	Isolation of RNA	10 preps 50 preps 250 preps	78772 78773 78774
PrepEase® Plant RNA Spin Kit	Isolation of RNA from plant cells	20 preps 50 preps	78770 78771
PrepEase® RNA/Protein Spin Kit	Isolation of RNA/protein	10 preps 50 preps 250 preps	78870 78871 78872
rDNase I, RNase-Free	Removal of contaminating DNA	1,000 units 2,500 units	78411
TE Buffer, 1X Solution	Resuspension/dilution of DNA	100 ml 10 x 1 ml 500 ml	75893

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Material Safety Data Sheet

Revision: 04/13/2009

Hazard information is provided for compliance with both the UK Chemicals (Hazard Information and Packaging) (CHIP) Regulations and the US Hazard Communication Standard (HCS)

IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND COMPANY	PRODUCT NAME:	PRODUCT CODE:	EEC NUMBER:
	Poly(A) Tail-Length Assay Kit	76450	None

SUPPLIER:
USB® Products - Affymetrix, Inc.
26111 Miles Road, Cleveland, Ohio 44128 Phone: (216) 765-5000
Please visit our website at **www.usbweb.com** for contact information on USB product distributors within your area.

EMERGENCY CONTACT:
Chemtrec: (800) 424-9300
Outside USA & Canada: 703-527-3887

COMPOSITION/ HAZARDOUS COMPONENTS	HAZARD	CAS NO.	%WT	TLV	CHIP R & S Phrases
	For Component 76463:				R:36/37/38 Irritating to eyes, respiratory system and skin. S:23 Do not breathe vapour. S:24/25 Avoid contact with skin and eyes. S:36/37 Wear suitable protective clothing and gloves.
	Tris-HCl	1185-53-1	~3.9%	—	
	Potassium Chloride	7447-40-7	~2.8%	—	
	For Component 71195:				
	Tris-HCl	1185-53-1	> 1%	—	
	Potassium Chloride	7447-40-7	> 1%	—	
	For Component 76465:				
	Potassium Chloride	7447-40-7	~1.9%		See information above.
	For Components 76461 and 76464:				R:36/37/38 Irritating to eyes, respiratory system and skin. S:26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S:36/37 Wear suitable protective clothing and gloves.
	Glycerol	56-81-5	~50%	See "Regulatory Information" Section	
	For Component 76460:				
	Tris-HCl	1185-53-1	~1.6%	—	
	Glycerol	56-81-5	~25%	—	
	For Component 75788:				No applicable information.
	HeLa Total RNA	N/A	~100%	—	

HAZARDS IDENTIFICATION

CHIP
Biohazard; Irritant
HCS
Biohazard; Irritant

FIRST-AID MEASURES

EYES: Flush with water for 15 min. Seek medical advice if irritation persists.
SKIN: Flush with water, then wash thoroughly with soap and water. Remove contaminated clothing and wash before reuse. Seek medical attention if irritation persists.
INHALATION: Remove the victim from exposure and move to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Keep victim quiet and warm. Seek immediate medical attention.
INGESTION: Drink water and seek immediate medical attention. Avoid alcoholic beverages. Never give anything by mouth to an unconscious person.

FIRE-FIGHTING INFORMATION

Use media suitable to extinguish the supporting or surrounding fire. Wear NIOSH (or equivalent) approved self contained breathing apparatus. For small fires only: use carbon dioxide, dry powder or foam. Emits toxic fumes under fire conditions. For Glycerol: Contact with strong oxidizing agents may produce an explosion.
Explosion Limits for Glycerol = Lower - 1.1; Upper - Not available.
Flash point for Glycerol = 193°C (379.4°F); Autoignition temperature for Glycerol = 400°C (752°F).

ACCIDENTAL RELEASE MEASURES

Caution: Catalog# 75788 is isolated from human sources. Handle all products prepared from human sources as if they were capable of transmitting infectious agents. Avoid accidental inoculation, intravenous injection or contact with open wounds. Wash thoroughly after handling. Observe universal precautions when working with this product. Wear appropriate personal protective equipment and clothing including lab coat, safety glasses, gloves and NIOSH-approved (or equivalent) respirator appropriate for the hazard. Contain the spill with an inert absorbent and place in a suitable waste container. Avoid contact of material with skin or eyes. Use adequate ventilation.

HANDLING AND STORAGE

Caution: Catalog# 75788 is isolated from human sources. Handle all products prepared from human sources as if they were capable of transmitting infectious agents. Avoid accidental inoculation, intravenous injection or contact with open wounds. Wash thoroughly after handling. Observe universal precautions when working with this product. Wear appropriate personal protective equipment and clothing including lab coat, safety glasses, gloves and NIOSH-approved (or equivalent) respirator. A qualified industrial hygienist should evaluate the need for respiratory protection. Use adequate ventilation. Avoid contact of material with skin or eyes. Store kit at -20°C away from incompatible materials.

PERSONAL PROTECTION

Caution: Catalog# 75788 is isolated from human sources. Handle all products prepared from human sources as if they were capable of transmitting infectious agents. Avoid accidental inoculation, intravenous injection or contact with open wounds. Wash thoroughly after handling. Observe universal precautions when working with this product. Wear appropriate personal protective equipment and clothing including lab coat, safety glasses, gloves and NIOSH-approved respirator. A qualified industrial hygienist should evaluate the need for respiratory protection. Use respiratory protection approved by NIOSH (or equivalent) and appropriate to the hazard. Avoid contact of material with skin or eyes. Mechanical ventilation or local exhaust as needed to control exposure to dust, vapors or mists. Access to a safety shower and eye-wash.

PHYSICAL AND CHEMICAL PROPERTIES

Appearance: Kit containing vials of solutions
Boiling Point: No data available
Vapor Pressure: No data available
Vapor Density: No data available
Solubility (Water): All components are soluble
Specific Gravity: No data available
Percent Volatile: No data available
Evaporation Rate: No data available
Chemical Formula: Not applicable

STABILITY AND REACTIVITY

Product is stable under normal conditions. Avoid prolonged excessive heat which may cause decomposition. Store away from strong bases, strong acids, and strong oxidizing agents. Hazardous decomposition products may include carbon oxides. Hazardous polymerization will not occur. For Glycerol: Avoid strong oxidizing agents including mixtures with hydrogen peroxide, potassium permanganate, trifluorobromide, calcium hypochlorite, nitric acid, sulfuric acid, perchloric acid and lead oxide. Contact with Sodium Hypochlorite and Hypochlorous acid may cause an explosion.

TOXICOLOGICAL INFORMATION

EFFECTS OF OVEREXPOSURE:
EYES: Contact may cause irritation.
SKIN: Contact may cause redness, swelling and pain at any site, especially mucous membranes.
INHALATION: Excessive inhalation of vapor may cause irritation, cough and shortness of breath.
INGESTION: Ingestion or excessive exposure may lead to nausea, vomiting and diarrhea. Large amounts may cause weakness, collapse and coma.
TARGET ORGANS: Eyes and Skin.
ADDITIONAL INFORMATION:
Tris-HCl - RTECS: No data available.
Potassium Chloride: Irritation, mutation and toxicity data listed in RTECS under TS8050000.
Irritation data: Eye Rabbit 500 mg/24H = Mild (1972).
Toxicity data: Oral Rat LD50 = 2600 mg/kg (1972).
Laboratory experiments have resulted in mutagenic effects.
Glycerol: Irritation, mutation, reproductive effects and toxicity data listed in RTECS under MA8050000.
Irritation data: Eye Rabbit 500 mg/24H = Mild (1986). Skin Rabbit 500 mg/24H = Mild (1986).
Toxicity data: Oral Rat LD50 = 12600 mg/kg (1945). Inhalation Rat LC50 = >570 mg/m3/1H (1970).
CAUTION: Catalog# 75788 is isolated from human sources. Handle all products prepared from human sources as if they were capable of transmitting infectious agents. Avoid accidental inoculation, intravenous injection or contact with open wounds. Wear appropriate personal protective equipment. Wash thoroughly after handling. Observe universal precautions when working with this product.
Definition(s): RTECS = Registry of Toxic Effects of Chemical Substances.
ACGIH = American Conference of Governmental Industrial Hygienists.
OSHA = Occupational Safety and Health Administration.

ECOLOGICAL INFORMATION

No information available.

DISPOSAL CONSIDERATIONS

Dispose of material in accordance with applicable local, state, and federal regulations.

TRANSPORTATION INFORMATION

US DOT / IATA: No applicable information.

REGULATORY INFORMATION

RCRA - No applicable information.
SARA 302 - No applicable information.
SARA 313 - No applicable information.
EPA TSCA Section 8(b) - For Glycerol, Tris-HCl, and Potassium Chloride: Chemical Inventory.
Exposure Limits - For Glycerol: ACGIH TLV-TWA 10 mg/m3 (total particulate).
OSHA PEL TWA: 15 mg/m3 (total dust).
California Proposition 65 - No applicable information.

This data sheet is based upon information believed to be reliable. The Company makes no statement or warranty as to the accuracy or completeness of the information contained herein which is offered for your consideration, investigation and verification. Any use of the information contained in this data sheet must be determined by the user to be in accordance with appropriate applicable regulations.