# USB® Poly(A) Tail-Length Assay Kit

**Product number 76455**
**5 G/I tailing, 20 RT, and**
**80 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 80 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 | 500 µl |
| Universal PCR Reverse Primer, 10 µM | 82 µl |
| HotStart-IT® Taq DNA Polymerase, 1.25 units/µl | 2 x 50 units |
| Control, human actin PCR Forward Primer, 10 µM | 8 µl |
| Control, HeLa Total RNA, 100 ng/µl | 10 µl |
| $MgCl_2$, 25 mM | 1 ml |
| Water, Nuclease-Free | 2 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 16 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 minutes incubation)
- Step 2: Reverse transcription (70 minutes incubation)
- Step 3: PCR amplification (30-60 minutes incubation)
- Step 4: Detection

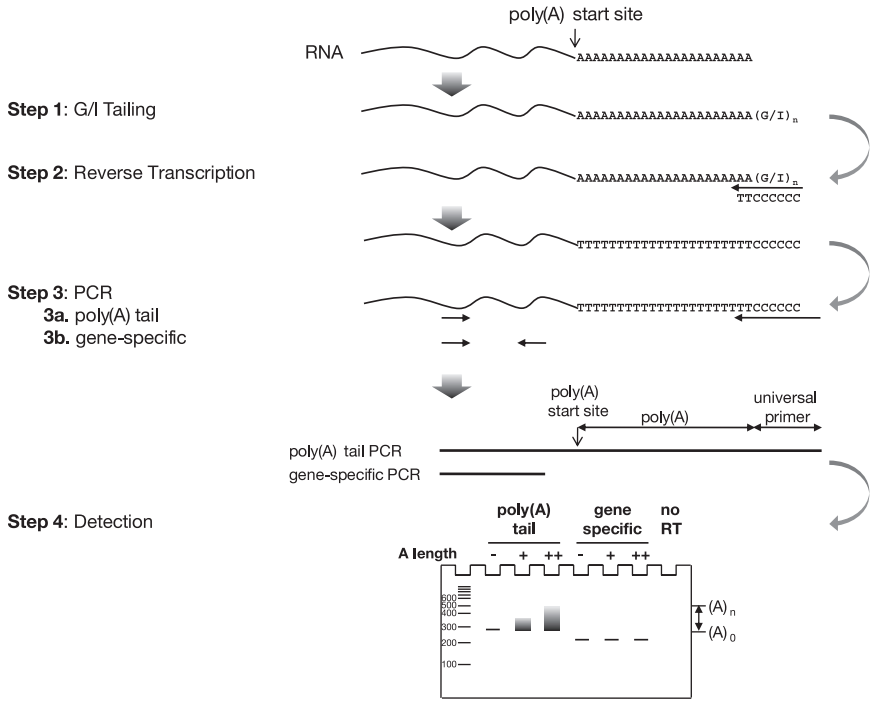



**Figure 1. Poly(A) tail-length assay procedure.**

## 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 (PN 76712 or 76710)
  - DNA loading buffer (PN 76715 or 76720)
  - 2-2.5% agarose (PN 32802) gel and TAE buffer (PN 75904 or 74015)
  - 4-6% non-denaturing polyacrylamide (PN 75848) and TBE buffer (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 RNase *AWAY*® 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 78411). 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 minutes
2. Reverse transcription: 44°C for 60 minutes; 92°C for 10 minutes; and 4°C hold
3. PCR amplification:

| Two-step PCR, **recommended** | Three-step PCR |
|---|---|
| 94°C for 2 minutes<br>30-35 cycles of:<br>94°C for 10 seconds<br>60°C for 30-60 seconds<br>72°C for 5 minutes<br>4°C hold | 94°C for 2 minutes<br>30-35 cycles of:<br>94°C for 10 seconds<br>58°C for 30 seconds<br>72°C for 30 seconds<br>72°C for 5 minutes<br>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 minutes
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 minutes; 92°C for 10 minutes; 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 |
| 25 mM $MgCl_2$* | 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. If a "No RT negative control" reaction is performed it should have no signal. Examples of results are shown in Figs. 3 and 4.

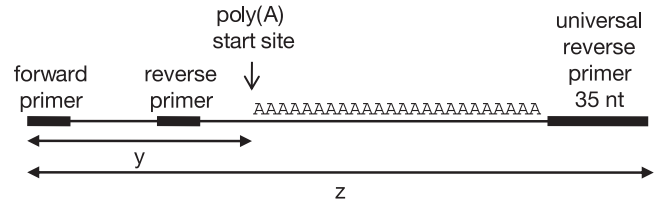



**Figure 2. Example of poly(A) tail-length determination.** A-tail length is (z-y-35) where z can vary based on gel results.

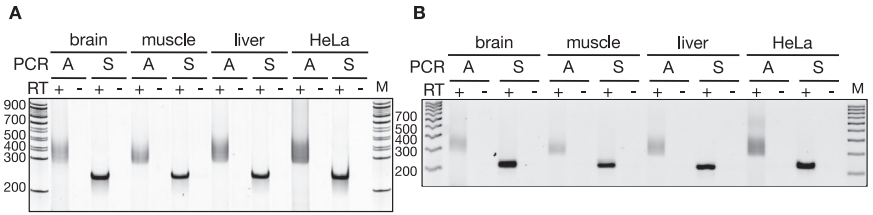



**Figure 3. Comparison of human actin poly(A) tail-lengths in brain, muscle, liver and HeLa cell.** One microgram total RNA and 4 µl of diluted RT samples were used in G/I tailing and PCR reactions, respectively. The recommended two-step PCR program was used. One half of each PCR reaction (12.5 µl) was 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 (PN 76712) (M).

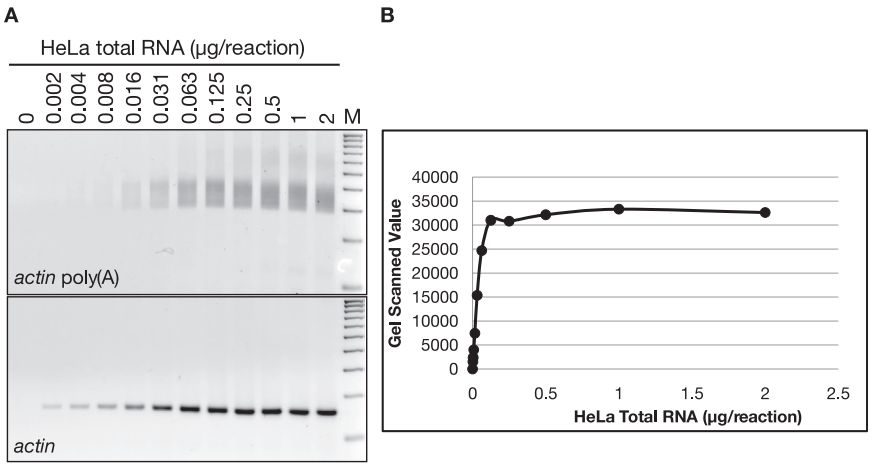



**Figure 4. Detection sensitivity of the USB Poly(A) Tail-Length Assay.** Actin poly(A) tail-length was determined from a two-fold serial dilution HeLa total RNA. Samples were processed as described in Fig. 3B (**A**). The left image was quantified by densitometry (**B**).

## 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 10 µM and used at a final concentration of 400 nM.

Gene-specific forward and reverse primers: These are the primers that are user-defined for the gene-of-interest. They should be diluted to 10 µM in TE Buffer (PN 75893) and used at a final concentration of 400 nM. 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:

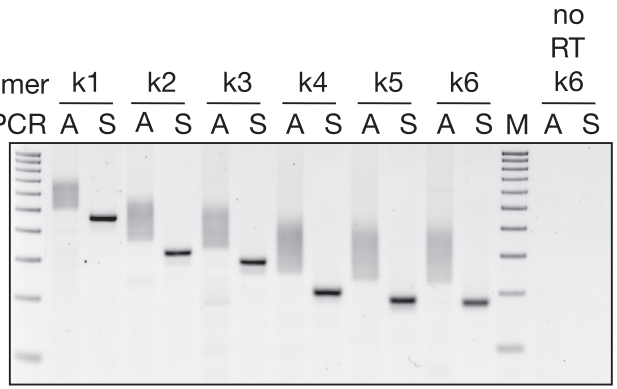
NCBI, http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome
Primer3, http://frodo.wi.mit.edu
IDT, http://www.idtdna.com/Scitools/Applications/Primerquest

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**

```
          k1
4981 ccccacagag ctaactgggt tacagtgttt tatccgaaag tttccaattc cactgtcttg

5041 tgttttcatg ttgaaaatac ttttgcattt ttcctttgag tgccaatttc ttactagtac
          k2                                                  k3
5101 tatttcttaa tgtaacatgt ttacctggaa tgtattttaa ctatttttgt atagtgtaaa
                    k4
5161 ctgaaacatg cacattttgt acattgtgct ttcttttgtg ggacatatgc agtgtgatcc
               k5   k6
5221 agttgttttc catcatttgg ttgcgctgac ctaggaatgt tggtcatatc aaacattaaa

5281 aatgaccact cttttaattg aaattaactt ttaaatgttt ataggagtat gtgctgtgaa

5341 gtgatctaaa atttgtaata tttttgtcat gaactgtact actcctaatt attgtaatgt
                    RP  poly(A) start
5401 aataaaaata gttacagtga caaaaaaaaa aaaaaa
```

**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 (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 minutes.
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:<br>10% ammonium persulfate (PN 76322) in water<br>TEMED (PN 76320) | <br>120 µl<br>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 minutes.
4. Prepare sample by adding loading buffer to 1X (*e.g.* 4 µl of 6X Loading Buffer, PN 76715 or 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 minutes.
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<br>– Check RNA integrity by gel electrophoresis or bioanalyzer.<br>2. Low abundant RNA target<br>– Increase the amount of total RNA to 2 µg per G/I tailing reaction.<br>– Use poly(A)-enriched RNA. Up to 0.5 µg poly(A) RNA sample per reaction can be used.<br>– Increase the sample volume for gel analysis.<br>3. Sub-optimal PCR condition<br>– Increase the amount of diluted RT to 5 µl per PCR reaction.<br>– Optimize $MgCl_2$ for the PCR reaction.<br>– Try different PCR forward primer.<br>– Increase the number of PCR cycles.<br>– Decrease PCR annealing temperature.<br>– Increase PCR extension time.<br>– Try the three-step PCR protocol.<br>– Use the supplied PCR reagents. These components have been optimized for use with this assay. |
| **Non-specific signal** | 1. Poor RNA sample quality<br>– 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.<br>2. Isoform detection<br>– Check if the gene-of-interest has different isoforms and the unexpected signals correspond to the presence of alternatively spliced transcripts.<br>– Design new specific forward primers that allow isoform discrimination. |

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

If problems persist please contact Technical Support for assistance at (888) 362-2447 or USBtechsupport@affymetrix.com. For technical support outside the U.S., please visit our website for up-to-date contact information within your area.

## References


1. Parker, R., and Song, H. (2004) *Nat. Struct. Mol. Biol.* **11**, 121-127.
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 |
|---|---|---|---|
| Agarose LE | Gel electrophoresis | 25 gm<br>100 gm<br>250 gm<br>500 gm<br>1 kg | 32802 |
| 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<br>5 ml | 76715 |
| 6X DNA Loading Buffer (BXF) | Gel electrophoresis | 1 ml<br>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<br>5 L | 75904 |
| TAE Buffer, 50X Solution | Gel electrophoresis | 100 ml | 74015 |
| TBE Buffer, 5X Solution | Gel electrophoresis | 1 L<br>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 | 50 preps<br>250 preps | 78766<br>78767 |
| PrepEase Plant RNA Spin Kit | Isolation of RNA from plant cells | 50 preps | 78771 |
| PrepEase RNA/Protein Spin Kit | Isolation of RNA/protein | 50 preps | 78871 |
| rDNase I, RNase-Free | Removal of contaminating DNA | 1,000 units<br>2,500 units | 78411 |
| TE Buffer, 1X Solution | Resuspension/dilution of DNA | 100 ml<br>10 x 1 ml<br>500 ml | 75893 |

## Affymetrix, Inc.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