RNA/cDNA INSPECTOR KIT
Product Code **INSP-1**

Technical Bulletin No. MB-740

ProductInformation

TECHNICAL BULLETIN

Product Description

The RNA/cDNA Inspector Kit is designed to evaluate RNA integrity and quality, evaluate successful cDNA synthesis, and determine relative RNA abundance. The kit provides nine oligonucleotide PCR primer sets complementary to different sequences within three different "housekeeping" genes. The human guanine nucleotide exchange factor (also known as p619)[1], β-actin[2,3], and glyceraldehyde 3-phosphate dehydrogenase (GAPDH)[4] genes are conserved in human, rat and mouse and ubiquitously expressed in many different tissues and cell lines.

The kit is designed to evaluate RNA and cDNA for the presence of transcripts from 1 kb to 14 kb long. The p619 PCR primer sets amplify sequences located in intervals of 1000-2000 bases along the 15 kb DNA coding sequence of the p619 gene. These PCR products form a 100 bp size ladder which is easily visualized. The PCR primers of the β-actin and the GAPDH genes are complementary to sequences within the coding sequence of these genes. The β-actin and GAPDH primers sets generate amplification products of approximately 450 and 350 respectively.

The RNA/cDNA Inspector kit is based on PCR† amplification of cDNAs and has been tested with RNA from human, mouse and rat. When testing RNA quality, full length first strand cDNA should be prepared before PCR amplification is performed. The cDNA is then amplified using any or all of the supplied primer sets and the size of the PCR product is evaluated. Each primer set is indicative of a certain RNA (from poly A site) or cDNA length. Using RNA or cDNA preparations, these sets will give PCR products of the sizes summarized in Table 1 which are indicative of the respective transcript sizes.

The PCR products may also be labeled and used as probes for the evaluation of RNA quality by Northern blotting. The tissue expression profile of p619 and β-actin is similar. However, as the level of expression of the p619 gene is 30-70 times less than β-actin, it is possible to use the PCR products of any of the sets 1-7 and that of β-actin to probe RNA in Northern blots to evaluate presence of low and high gene expression respectively.

Reagents Provided

Each primer set is sufficient for 50 reactions.
Sequences are 5' →3'.

p619 gene PCR Primers sets
(GenBank Accession No. U50078)

- p619 Primer Set 1, Product Code P 1855 1 vial
sense 12984, GGC AGT TGG AGC TGA ACA CA
antisesnse 13892, TGG AGG TCC AGA GGC TTC TT

- p619 Primer Set 2, Product Code P 1980 1 vial
sense 9406, AAT GAC CGG CGC ATT GTA CC
antisense 10202, GCA TTA GCC AAC TCC AGA GG

- p619 Primer Set 3, Product Code P 2105 1 vial
sense 8290, GCT AAC CGC ACA GCC TTG TC
antisense 8998, GCC AGG TAG GCC AAT CCA GT

- p619 Primer Set 4, Product Code P 2230 1 vial
sense 5194, AGT GGC CGA TTG CAT CAC TA
antisense 5802, GAG TTC GGC TGC ATG TTG TT

- p619 Primer Set 5, Product Code P 2355 1 vial
sense 4191, TCC GGA GCC AGA GGA TGA AG
antisense 4690, GTC TGC CAC TCA GTG CGT AA

- p619 Primer Set 6, Product Code P 2480 1 vial
sense 2280, ACA TAG TCT GGC ATG GAC TG
antisense 2676, CCG TTC TCG TAA TGG AGG TA

- p619 Primer Set 7, Product Code P 2605 1 vial
sense 1029, TTC ATC TGC TGA TCG GAG TC
antisense 1329, TCC AGC TTC AAT GGT CTG TG

β-Actin gene PCR primer set
(EMBL Accession No. X00351)

- β-Actin Primer Set, Product Code P 2855 1 vial
sense 493, T(C/T)G TGA TGG ACT CCG G(A/T)G AC
antisense 945, C(G/A)C CAG ACA GCA CTG TGT TG

GAPDH gene PCR primer set
(GenBank Accession No. M33197)

- GAPDH Primer Set, Product Code P 2730 1 vial
sense 507, TGC (C/A)TC CTG CAC CAC CAA CT
antisense 856, (C/T)GC CTG CTT CAC CAC CTT C

Table 1

p619 Primer Sets	Sense Site	Anti-Sense Site	PCR Product Size (bp)	mRNA Transcript Length (bp)
Set 1	12,984	13,892	908	2,180
Set 2	9,406	10,202	796	5,760
Set 3	8,290	8,998	708	6,875
Set 4	5,194	5,802	608	9,970
Set 5	4,191	4,690	499	10,980
Set 6	2,280	2,676	396	12,890
Set 7	1,029	1,329	300	14,150

βActin Primer Set	Sense Site	Anti-Sense Site	PCR Product Size (bp)	mRNA Transcript Length (bp)
β-Actin	493	945	452	880

GAPDH Primer Set	Sense Site	Anti-Sense Site	PCR Product Size (bp)	mRNA Transcript Length (bp)
GAPDH	507	856	349	1,060

Figure 1

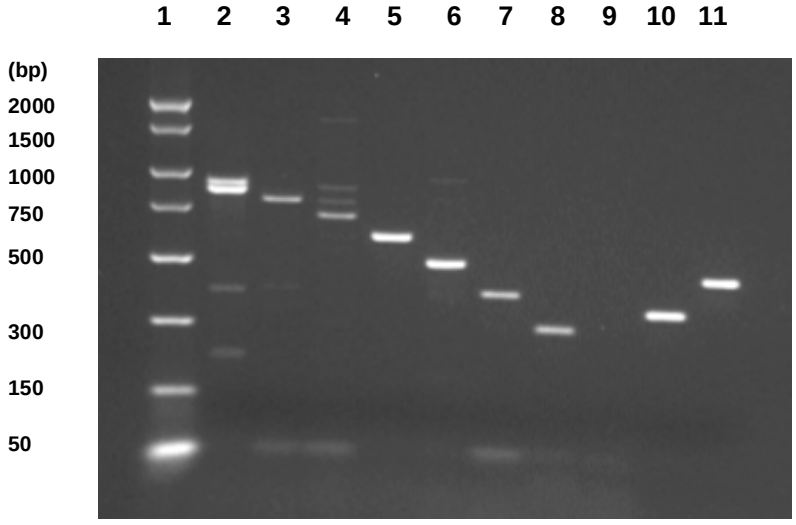

Figure 1. PCR products resolved on 1.5% standard agarose gel (A9539) following RT-PCR using total RNA from HEK293 cells. Lane 1, PCR Marker (Product Code P 9577), Lanes 2-8, p619 PCR primer sets 1-7; Lane 9, Negative control (no RT), Lane 10, GAPDH PCR primer set; Lane 11, β-Actin PCR primer set.

Reagents and Equipment Required but Not Provided
(Sigma product numbers have been given where appropriate)

- Water, PCR Reagent, Product Code W 1754
- GenElute™ Mammalian Total RNA Purification Kit, Product Codes RTN10, RTN70 and RTN350 **or**
- TRI Reagent, Product Code T 9424
- Sample RNA for transcription and amplification
- Enhanced Avian First Strand Synthesis Kit containing enhanced avian reverse transcriptase, 10X buffer, RNase inhibitor, and anchored oligo$(dT)_{23}$ primer, Product Code STR-1 **or**
  - M-MLV reverse transcriptase, Product Code M 1302
  - 5X Reverse transcriptase buffer, Product Code B 0175, provided with M 1302
  - RNase inhibitor, Product Code R 2520
  - Oligo$(dT)_{12\text{-}18}$ primer, Product Code O 6378
- Thermal cycler
- 0.5 or 0.2 ml thin-walled PCR tubes, Product Codes P 3114 and P 3364
- Deoxynucleotide mix (10 mM each dNTP), Product Code D 7295
- 10X PCR buffer, Product Code P 2192
- JumpStart *Taq* DNA Polymerase, Product Codes D 9307 or D 4184
- Agarose, molecular biology grade, Product Code A 9539
- 10X TAE buffer, Product Code T 9650
- Step ladder DNA marker, Product Code S 7025
- Ethidium bromide, Product Code E 1510

Precautions and Disclaimer
Sigma's RNA/cDNA Inspector Kit is for R&D use only. Not for drug, household or other uses.

Preparation Instructions
Reconstitute each primer set with 50 µl of PCR grade water. Mix gently by vortexing and centrifuge briefly to collect the solution at the bottom of the tube.

Storage
Store at –20 °C. Repeated freezing and thawing or storage in "frost-free" freezers are **not** recommended.

Procedure
The procedure given below is for beginning with total RNA. If cDNA is used, begin with Section IV. The optimal concentrations of reverse transcriptase, JumpStart *Taq* polymerase, template RNA, primers and amplification parameters will depend on the system used and should be determined empirically.

I. Total RNA Preparation
For effective isolation of total RNA, use the GenElute™ Mammalian Total RNA Purification Kit or TRI Reagent and follow the instructions provided.

II. First Strand cDNA Synthesis using the First Strand Synthesis Kit

1. Combine the following components in a 0.2 ml or 0.5 ml microcentrifuge tube:

RNA	2.5 µg
Anchored Oligo $(dT)_{23}$	1 µl

2. Heat at 95 °C for 45 seconds.

3. Immediately add cold PCR grade water for a final volume of 10 µl and keep on ice.

4. Add the following reagents:

RNase Inhibitor (20 units/µl)	1 µl
10X Reverse Transcriptase Buffer	2 µl
eAMV Reverse Transcriptase	1 µl
dNTP mix	2 µl
PCR grade water	q.s.
Total Volume	20 µl

5. Incubate at 47 °C for 1 hour.

6. Inactivate the reverse transcriptase by incubating the reaction at 95 °C for 5 minutes.

7. Use immediately or store at –70 °C.

III. First Strand cDNA Synthesis using M-MLV RT

1. Combine the following components in a 0.2 ml or 0.5 ml microcentrifuge tube:

RNA	10 µg
Oligo $(dT)_{12-18}$ (1.5-2 µg/µl)	4 µl

2. Heat at 95 °C for 45 seconds.

3. Add immediately 30 µl cold PCR grade water and keep on ice.

4. Add the following reagents:

RNase Inhibitor (40 units/µl)	2 µl
5X Reverse Transcriptase Buffer	20 µl
M-MLV Reverse Transcriptase	3 µl
dNTP mix	10 µl
100 mM DTT	10 µl
PCR grade water	q.s.
Total Volume	100 µl

5. Incubate at 37 °C for 1 hour.

6. Inactivate the enzyme by incubating the reaction at 95 °C for 5 minutes.

7. Use immediately or store at –70 °C.

IV. PCR amplification

1. For each primer set assemble the following PCR mixture:

cDNA (from First Strand cDNA Synthesis)	1 µl
Primer set	1 µl
10X PCR buffer	2.5 µl
JumpStart *Taq* DNA polymerase	1 µl
dNTP Mix	0.5 µl
PCR grade water	19 µl
Total Volume	25 µl

Note: If 50 µl reactions are preferred, double the reagent volumes and optimize the thermal cycling conditions.

2. 30 cycles using the following cycling parameters are recommended:

Initial denaturation/ RT Inactivation	94 °C for 3 min
For cycle 1-29	
Denaturation	94 °C for 30 sec
Annealing	55 °C for 30 sec
Extension	72 °C for 1 min
Final Extension	72 °C for 10 min
Hold	4 °C

3. Evaluate the amplified DNA by agarose gel electrophoresis. Typical results are shown in Figure 1.

References

1. Rosa, J. L., et.al., EMBO J., **15**, 4262-4273 (1996)
2. Schliwa, M., *Cell Biology Monographs*, Vol. 13 "The Cytoskeleton, an Introductory Survey," Springer-Verlag, 1986
3. Herman, I. M., Curr. Opin. Cell Biol., **5**, 48-55 (1993)
4. Ishitani, R., and Chung, D. M., Proc. Natl. Acad. Sci., USA, **93**, 9937-9941 (1996)

Related Products

RNA Isolation

GenElute™ Mammalian Total RNA Purification Kit, for isolating total RNA from tissue or cells, Product Codes RTN10, RTN70 and RTN350

PCR Products

Product Code D 9307, JumpStart Taq DNA Polymerase with 10X reaction buffer containing $MgCl_2$
Product Code D 4184, JumpStart Taq DNA Polymerase with 10X reaction buffer without $MgCl_2$

RT Products

Product Code STR-1, Enhanced Avian First Strand Synthesis Kit for reverse transcription
Product Code A 4464, Enhanced Avian Reverse Transcriptase

†The PCR process is covered by patents owned by Hoffman-LaRoche, Inc.

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