

Figure 1.
Broad Temperature Range of AffinityScript One-Step RT-PCR Kit

Ten nanograms of mouse liver total RNA was amplified with the AffinityScript One-Step RT-PCR kit with primers specific for a 1.6 kb amplicon of the mouse GPT gene. The initial incubation was performed at 37, 45, 50, 55 or 60°C for 30 minutes followed by a 2 minute denaturation step at 95°C and 40 cycles of 95°C for 20s, 58°C for 20s and 72°C for 72s. 1 µl of PCR product from each RT-PCR reaction was run on the Agilent Technologies 2100 Bioanalyzer. Data is presented in the form of a gel (A) and the corresponding electropherogram (B).

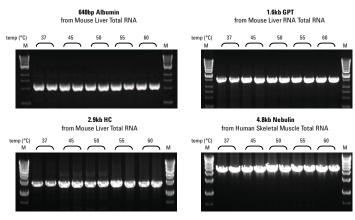


Figure 2.
Broad Temperature Range of AffinityScript One-Step RT-PCR Kit

Temperatures for RT were varied from $37-60^{\circ}\text{C}$ for 5 minutes (Alb), 15 minutes (GPT), or 30 minutes (Complement and Nebulin); followed by 1 minute denaturation at 95°C and 40 cycles of 95°C for 20s, 58 or 60°C for 20s and 68°C for 45s per kb product respectively. A final 3 minute extension at 68°C followed. Lane M: 1 Kb DNA ladder.

AffinityScript One-Step RT-PCR Kit

Looking for unsurpassed performance from a one-step RT-PCR kit? You just found it!

Data Sheet

The AffinityScript One-Step RT-PCR Kit outperforms the competition with unparalleled yield, sensitivity and specificity. The AffinityScript One-Step RT-PCR Kit provides a complete system for fast, high-yield, reliable single-tube RT-PCR. The master mix format, coupled with robust performance and rapid cycling conditions, makes this system ideal for gene profiling and other high-throughput end-point RT-PCR applications.

With AffinityScript One-Step RT-PCR, you can:

- Achieve full length, high fidelity RT-PCR from targets up to 9.5 kb in length
- Obtain superior PCR yield due to the enhanced processivity of Herculase II fusion DNA polymerase and the ArchaeMaxx PCR enhancing factor
- · Overcome secondary structure in difficult and GC-rich targets
- Perform the RT step at a temperature of your choosing
- Amplify multiple targets in one tube at the same time

AffinityScript One-Step RT-PCR Kit utilizes the multiple temperature reverse transcriptase (RT) which shows optimal activity over a broad temperature range, from 42°C to 55°C, allowing greater flexibility in first-strand synthesis conditions (Figure 1). The AffinityScript RT provides excellent cDNA yields, full-length cDNAs, and superior RT performance for both routine and challenging RNA samples. The cDNA is amplified in the same tube by Herculase II fusion DNA polymerase, a highly processive *Pfu*-based DNA polymerase containing a high affinity double-stranded DNA binding domain.

For full-length RT-PCR of long templates, the AffinityScript One-Step RT-PCR Kit demonstrates superior yield and length compared to the competition (Figure 2).



AffinityScript RT routinely detects and amplifies targets using 1 ng of total RNA (Figure 3).

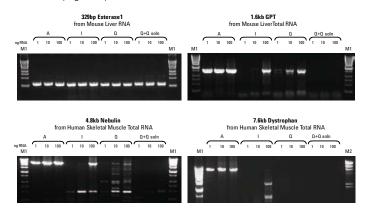


Figure 3
Comparison of One-Step RT-PCR systems

Total RNA (1, 10 and 100 ng) was amplified for each target using manufacturers recommended protocol. A- AffinityScript One-Step RT-PCR Kit; I - Competitor I; Q - Competitor Q; Q+Q soln - Competitor Q with Q solution added. M1: 1 Kb DNA ladder; M2: lambda/*Hind*II marker.

The kit combines the high-performance of AffinityScript RT with Herculase II fusion technology to produce better yield specificity than other one-step RT-PCR kits (Figure 4).

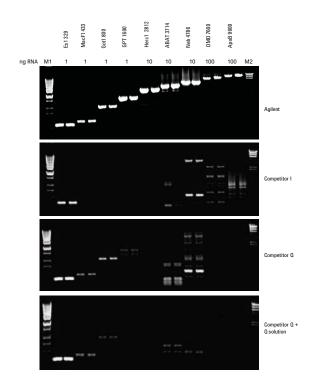


Figure 4
Increased Sensitivity with the AffinityScript One-Step RT-PCR Kit

Total RNA (1, 10 and 100 ng) was amplified for each target using manufacturers recommended protocol. A- AffinityScript One-Step RT-PCR Kit; I — Competitor I; Q — Competitor Q; Q+Q soln — Competitor Q with Q solution added. M1: 1 Kb DNA ladder; M2: lambda/Hindll marker.

You can even detect multiple targets of interest to be amplified simultaneously (Figure 5) saving time and reagents.

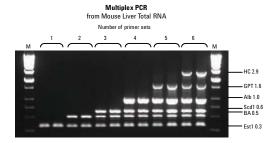


Figure 5
AffinityScript RT allows Amplification of Multiple Targets Simultaneously

One to 6 targets were amplified using the AffinityScript One-Step RT-PCR Kit. Group 1:Est1 – Esterase; group 2: BA – β-actin; group 3: Scd1 - stearoyl-Coenzyme A desaturase 1; group 4: Alb – Albumin; group 5: GPT - glutamic pyruvic transaminase; group 6: 1HC – hemolytic complement; M: 1 Kb DNA ladder.

For your added peace of mind, AffinityScript One-Step RT-PCR Kit contains a recombinant RNase inhibitor to minimize RNA degradation.

Description	Quantity	Reactions	Catalog No.
AffinityScript One-Step RT-PCR Kit	1	100	600188

Learn more:

http://www.stratagene.com/products/showProduct.aspx?pid=887

Find an Agilent customer center in your country:

www.agilent.com/chem/contactus

U.S. and Canada

1-800-227-9770 agilent inquiries@agilent.com

Asia Pacific

inquiry lsca@agilent.com

Europe

info agilent@agilent.com

This item is intended for Research Use Only. Not for use in diagnostic procedures. Information, descriptions and specifications in this publication are subject to change without notice.

Agilent Technologies shall not be liable for errors contained herein or for incidental or consequential damages in connection with the furnishing, performance or use of this material.

© Agilent Technologies, Inc. 2010 Printed in the USA, January 30, 2010 5990-5213EN

