

	Made in USA
Catalog Number	600109
Product Name	AffinityScript Multiple Temperature Reverse Transcriptase, 200 Reactions
Quantity	AffinityScript Multiple Temperature Reverse Transcriptase, 200 µl (200 reactions) 10× AffinityScript RT Buffer, 1 ml 100 mM DTT, 800 µl
Certified By	Derek Hall
Quality Controlled By	Tricia Molina
Shipping Conditions	Shipped on dry ice.
Storage Conditions	Store at -20°C upon receipt.
Description	AffinityScript Multiple Temperature Reverse Transcriptase is a genetically engineered version of MMLV reverse transcriptase that exhibits improved specific activity over a broad range of cDNA synthesis temperatures from 42°C to 55°C. In addition to being more versatile compared to other reverse transcriptases, AffinityScript reverse transcriptase produces higher cDNA yields and provides superior performance in RT-PCR.
Test Conditions	AffinityScript multiple temperature reverse transcriptase is tested by generating cDNA from various RNA templates, with products ranging from 0.5 to 9.0 kb in size.
Contamination Test Conditions	AffinityScript multiple temperature reverse transcriptase is tested for the absence of detectable endonuclease, exonuclease, and RNase activities.
First-Strand cDNA Synthesis Protocol	<p>To a nuclease-free microcentrifuge tube, add the following three components in order. The total volume of the input RNA template, water, and primers should equal 14.2 µl.</p> <p>1 ng-5 µg of total RNA or 1 ng-250 ng of poly(A)+ mRNA x µl of RNase-free water 500 ng of Oligo(dT) or 300 ng of random primers</p> <p>Incubate the mixture of RNA template, water, and primers at 65°C for 5 minutes. Slowly cool to room temperature (~10 minutes) to allow primers to anneal to RNA.</p> <p>Add the following four components to the mixture. The final reaction volume is 20 µl.</p> <p>2 µl of 10× AffinityScript RT buffer 2 µl of 100 mM DTT 0.8 µl of 100 mM dNTP mix 1 µl of AffinityScript Multiple Temperature Reverse Transcriptase</p> <p>Mix the reaction gently and incubate the reaction at 42-55°C for 1 hour. (If random primers are used, pre-incubate at 25°C for 10 minutes.)</p> <p>Inactivate the reaction at 70°C for 15 minutes. Place cDNA on ice for subsequent use as template in PCR.</p> <p>Notes <i>AffinityScript multiple temperature reverse transcriptase performs optimally over the full range of 42-55°C. Typically, 42°C is a good starting point. For RNAs containing secondary structure and other challenging targets, a synthesis temperature of 55°C may be used without loss of performance.</i></p> <p><i>Depending on the quality of the RNA preparation, it may be beneficial to add RNase Block to the reaction to prevent degradation of RNA by RNases (addition of 20 units at the time of reverse transcriptase addition is sufficient).</i></p>
10× AffinityScript RT Buffer	500 mM Tris-HCl (pH 8.3) 750 mM KCl 30 mM MgCl ₂
Storage Buffer	20 mM Tris-HCl (pH 8.0) 0.1 mM EDTA 1 mM dithiothreitol (DTT) 0.01% Igepal CA-630 0.1 M NaCl 50% (v/v) glycerol

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