

AffinityScript Multiple Temperature Reverse Transcriptase, 10 Reactions

p/n 600105



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.

Specifications

High Yield for GC-rich Sequences	Yes
Long-read	20 Kb
Multiple Temperature Activity	Yes
Number of Reactions	10
Reactions per Kit	10
Thermal Cycler Type	Any
Volume	10 µL

Materials

Product Name	Quantity
AffinityScript Multiple Temperature Reverse Transcriptase (10 reactions)	10 µL
10x AffinityScript RT Buffer	1 ml
100 mM DTT	40 µL

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

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 μ L.

	Quantity
Total RNA or poly(A)+ mRNA	1 ng to 5 μ g; 1 ng to 250 ng
RNase-free water	χ μ L
Oligo(dT) or Random primers	500 ng; 300 ng

Incubate the mixture of RNA template, water, and primers at 65°C for five minutes. Slowly cool to room temperature (about ten minutes) to allow primers to anneal to RNA.

Add the following four components to the mixture. The final reaction volume is 20 μ L.

	Quantity
10x Affinity Script RT buffer	2 μ L
100 mM DTT	2 μ L
100 mM dNTP mix	0.8 μ L
AffinityScript Multiple Temperature Reverse Transcriptase	1 μ L

Mix the reaction gently and incubate the reaction at 42 to 55°C for one hour. (If random primers are used, pre-incubate at 25°C for ten minutes.)

Inactivate the reaction at 70°C for 15 minutes. Place cDNA on ice for subsequent use as template in PCR.

Notes

AffinityScript multiple temperature reverse transcriptase performs optimally over the full range of 42 to 55°C. Typically, 42°C is a good starting point. For RNA containing secondary structure and other challenging targets, a synthesis temperature of 55°C may be used without loss of performance.

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).

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