Product Insert

G-Script cDNA Synthesis Kit

PUREGENE

Store at -20°C

Cat No.	Pack Size
PGK150-A	40 rxn
PGK150-B	100 rxn

Component	PGK150-A 40 rxn	PGK150-B 100 rxn
G-Script Enzyme Mix	40 µL	100 µL
5X Buffer	500 µL	500 µL
Oligo dT (500 ng/µL)	40 µL	100 µL
Random Hexamer (400 ng/µL)	40 µL	100 µL
dNTP Mix (5 mM each)	200 µL	200 µL
RT Accelerator	40 µL	100 µL

Description

G-Script cDNA Synthesis Kit supplies all the components required to perform a rapid, sensitive, and reproducible RT-PCR for the detection and analysis of all types of RNA.

G-Script Enzyme Mix includes G-Script Reverse Transcriptase, which is active at high temperatures, is highly sensitive and can generate long cDNA strands. This mix also contains RNase inhibitor to protect RNA templates from degradation. 5X Buffer, which has been optimized to improve reverse transcription across a wide range of templates. Oligo dT primers and Random Hexamers provide flexible RNA priming methods for cDNA synthesis. RT Accelerator is included to remove contaminating DNA, eliminating the need for DNAse I treatment. It degrades double stranded DNA during the transcription of RNA and is inactivated after 2 minutes at 95 °C.

G-Script Reverse Transcriptase is an RNA-dependent DNA polymerase with a significantly attenuated RNase H activity. It can synthesize long cDNA strands, up to 11 kb, at a temperature range of 42 °C to 57 °C. The recommended amount of total RNA to use is between 1 pg and 1 µg.

Storage Conditions

Store at -20°C until ready for use. Avoid repeated freeze-thawing.

Additional Info

The use of disposable gloves, RNase and DNase free filter tips and plastics is recommended.

RT Accelerator is not required if DNase I treatment is performed prior to qRT-PCR.

Tips before use

Thaw the reagents on ice. Mix and spin down the solutions before use to recover the maximum amount. Do not vortex the G-Script Enzyme Mix. Briefly centrifuge to avoid bubbles within the wells. Always include a no template control and a no enzyme control.

Protocol

1.Add add the following components for each 20 µl reaction:

Component	Volume	Final Conc.
5X Buffer	4 µL	1 X
dNTP Mix	2 µL	500 µM each
RNA primer*	1 µL	
RT Accelerator	1 µL	
G-Script Enzyme Mix	1 µL	
Template RNA	1-5 µL	1 ng
Water, nuclease-free	to 20 µL	
Total Volume	**20 µL**	

*It is recommended that RNA primers be added to the final 1X reaction as follows: 1 µL of oligo dT or 1 µL of random hexamers or 1 µL of a blend of random hexamers and anchored oligo-dT 3:1 (v/v) or gene-specific primer (to final concentration of 0.5 – 2 µM). oligo dT is not suitable for use with most prokaryotic RNA. In these cases, random hexamers or gene-specific primers are recommended.

Example of Reverse transcription cycling program:

	Temp.	Time	Cycles
cDNA synthesis	42°C	30 min	1 cycle
Inactivation	95°C	2 min	1 cycle

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals