

COSMO cDNA PLUS synthesis Kit

(WF-1020500X)

Overview

Using the latest innovations in reverse transcriptase biotechnology and buffer chemistry, the *COSMO* cDNA PLUS synthesis kit produces cDNA with unparalleled quality. In addition to the optimised buffer and reverse transcriptase, the concentration of random hexamers with anchored oligo(dT) primers is also optimised to aid in the accuracy of cDNA synthesis. Furthermore, the enzyme is not inhibited by either rRNA or tRNA.

Stages

- 1. Primer annealing
- 2. Extension
- 3. Enzyme inactivation
- 4. RNA removal (optional)

Features of COSMO cDNA PLUS synthesis Kit

- Accuracy The random hexamers with oligo(dT) primers anneal to the RNA aiding in the process of reverse transcription.
- **High yield** At Willowfort, continued innovations in buffer chemistry and enzymology have drastically improved the performance of the reverse transcriptase.
- Versatile The cDNA product can be used in a range of downstream molecular biology techniques.

Product information

	Catalog number		Size	
	WF10205005	50 r	50 reactions	
	WF10205006	100	100 reactions	
	Reagent	50 reactions	100 react	ions
	RT Enzyme Mix	50µl	100µl	
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Required materials

- Template RNA (4.0pg-400ng) 0.2mL PCR tubes
 - RNase (optional)
- Pipettes and tips
- Vortex

- Thermocycler
- Centrifuge

Ice

Reaction timings

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Once each reaction tube has been set up, cDNA synthesis will be complete in 20 minutes. The cDNA product will be ready to use for a variety of downstream applications

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Storage conditions

The reagents provided in the COSMO cDNA PLUS synthesis kit should be stored at - 20° C. Where possible stored reagents in conditions of non-frost-free.

Warning!

Please be advised to wear appropriate eyewear, clothing and gloves. Read the Safety Data Sheets (SDS) for any further safety information. When working with RNA, ensure RNase free working conditions.

Any further information

For further information and protocols, visit our website <u>https://willowfort.co.uk/</u>. For any additional support, please contact us through <u>https://willowfort.co.uk/contact-us</u>

For research use only. These products should not be used for diagnostics



Components of COSMO cDNA synthesis Kit

RT Enzyme Mix

- **Reverse Transcriptase Enzyme** produces complementary DNA (cDNA) from template RNA at an increased enzymatic kinetic speed.
- **RNase Inhibitor Enzyme** A high affinity RNase inhibitor used to prevent degradation of the template RNA during cDNA synthesis.

cDNA Reaction Buffer (5X)

- **Optimised buffer** the latest buffer chemistry ensures optimal enzyme performance to maximise yield and minimise errors.
- Random Hexamers with oligo(dT) primers the combination of Oligo(dT) primer and Random hexamers increases accuracy of annealing regardless of the length of transcript.
- dNTPs This is included in the COSMO buffer. The purity of dNTPs in all Willowfort products is ≥99%, to ensure maximum fidelity and speed. For longer sequences, additional dNTPs maybe required (not included, but additional vials can be purchased on our website).
- Storage buffer This is included in the buffer. 20mM for cDNA synthesis.
 Tris buffer pH 8.0, 100mM KCl, 0.1mM EDTA, 1mM 6. Once the reaction is complete, the newly DTT and glycerol to prevent enzyme degradation.

COSMO cDNA PLUS synthesis kit protocol

- 1. Allow reagents to thaw and place on ice. Mix all solutions well before preparing the master mix. Check for any signs of precipitation.
- 2. Use Table 1 as a guide to prepare the cDNA Master Mix. Mix well and briefly centrifuge.

Table 1: Reagents required per sample					
Reagents per reaction tube	Volume				
cDNA Reaction buffer (5x)	4μΙ				
Nuclease-free water	to 20µl				
RT Enzyme Mix	1µl				
Template RNA (4.0pg-400ng)	Volume varies				

- Aliquot Master mix into 0.2mL PCR tubes, before adding the template RNA. Use 4.0pg—400ng RNA for the best results.
- 4. Gently mix samples using a vortex and briefly centrifuge.
- 5. Use Table 2 as a guide to set up the thermocycler for cDNA synthesis.
- 5. Once the reaction is complete, the newly synthesised cDNA can be used for downstream applications. We recommend using 5µl of the cDNA from the reaction in a PCR or qPCR application.

Table 2: Recommended Thermal Profile for cDNA Synthesis kit

Stage	Temperature	Time			
Stage 1: Primer annealing	25ºC	*5mins			
Stage 2: Extension	55°C	15mins			
Stage 3: Inactivation (<i>Optional</i>)	80-85°C	5mins			
Stage 4: RNA removal (<i>Optional</i>)					
*If required, increase time to 10 minutes					

Downstream Applications after cDNA synthesis

- RT-PCR
- qPCR
- Second strand applications

Troubleshooting – For any troubleshooting queries, please visit our website <u>http://willowfort.co.uk/end-point-pcr/cosmo-cdna-kit</u>.

Any further information, please contact us, via <u>https://willowfort.co.uk/contact-us</u>.