

WISENT ADVANCED™ cDNA SYNTHESIS KIT

ORDERING INFORMATION:

Catalogue number	Quantity	specification
801-100-XR	25 REACTIONS	cDNA synthesis
801-100-AR	100 REACTIONS	cDNA synthesis

PRODUCT DESCRIPTION:

Advantages:

Unbiased representation of 5' and 3' mRNA transcript ends

Sensitive detection of low copy number transcripts

High cDNA yields from as little as 1 pg total RNA

Simple 2 tube system

Wisent cDNA Synthesis Kit uses the latest technology in reverse transcription buffer chemistry to enhance cDNA synthesis speed and yield with accurate transcript representation. The reverse transcriptase, buffer system and combination of random hexamers with anchored oligo(dT) allow for unbiased, efficient, sensitive cDNA synthesis.

High quality cDNA synthesis for downstream qPCR analysis is essential for successful expression studies. cDNA synthesis is affected by the reverse transcriptase, buffer systems, enhancers and priming strategy. The Wisent cDNA synthesis mix removes the need for user optimization of these critical factors.

Wisent RTase is both thermostable and extremely active. The enzyme is blended with RNase inhibitor.

The RTase is not inhibited by ribosomal and transfer RNAs, total RNA is an ideal substrate. The 5x cDNA synthesis mix can be used with up to 4.0µg total RNA. The relative concentrations of random hexamers and anchored oligo(dT) have been optimized for the generation of cDNA for use in real-time PCR experiments.

This mix is optimized to generate cDNA for downstream real-time PCR analysis.

PRODUCT STABILITY AND STORAGE:

On arrival store at -20°C.

Avoid prolonged exposure to light.

If stored correctly the kit will retain full activity for 12 months. It is stable at 4°C for 1 month and can go through 30 freeze/thaw cycles with no loss of activity.

DIRECTIONS FOR USE:

OUR KIT CONTAINS:

5 x cDNA mix: anchored oligo(dT), random hexamers, 15 mM MgCl₂, 5 mM dNTPs, enhancers and stabilizers. Do not add further enhancers or MgCl₂ to the reaction.

Note:

Template: For total RNA: use between 4 pg and 4.0 µg per reaction.

Incubation temperature: For the majority of applications (<65% GC), incubate at a temperature of 42°C for 30 minutes. If regions of interest contain high secondary structure (>65% GC) incubation temperatures of up to 55°C might be used.

REACTION SETUP:

1. Before starting, allow 5 x cDNA mix to thaw and briefly vortex
2. Prepare a mastermix, as follows: - Important: insert reagents in sequence as listed

<u>Add:</u>	<u>Final concentration:</u>
A. WISENT 5 x cDNA synthesis MIX: 4 µl	1X
B. 20 x RTase 1.0 µl	- ADD BEFORE RNA
C. Total RNA between 4 pg and 4 µg	

Add PCR grade water up to 20 µl final volume

3. No RT control set up (optional):

<u>Add:</u>	<u>Final concentration:</u>
A. WISENT 5 x cDNA synthesis MIX: 4 µl	1X
B. Total RNA between 4 pg and 4 µg	-USE EQUAL
	AMOUNT AS IN STEP 2

Add PCR grade water up to 20 µl final volume

INCUBATION AND ENZYME DENATURATION:

4. Incubate at 42°C for 30 minutes
5. Incubate at 85°C 10 minutes to denature RTase

