		Second Strand cDNA Synthesis Kit	
		dNTP Based	dNTP/dUTP Based
Part No.	**Components**	**G475**	**G476**
E007	*E. coli* DNA Ligase (10 U/µl)	100 µl	100 µl
G469	*E. coli* DNA Polymerase I (10 U/µl)	100 µl	100 µl
E018	RNase H *E. coli* (5 U/µl)	100 µl	100 µl
RT-5-A	dNTPs Mixture (10 mM)	40 µl	-
RT-11-A	dNTP/dUTP Mixture (10 mM)	-	40 µl
RT-12-A	10X Second Strand cDNA Synthesis Buffer	200 µl	200 µl
	Size	25 rxns	

Product Description

Second Strand cDNA Synthesis Kit is an efficient system of generating double stranded cDNA from first strand cDNA as a template. The *E. coli* RNase H nicks the RNA in the DNA:RNA hybrid, while the *E. coli* DNA Polymerase replaces the RNA with deoxyribonucleotides. The *E. coli* DNA Ligase completes the double stranded DNA formation by linking the gaps between the newly synthesized cDNA strand. The dNTP based kit (Cat. No. G475) and dNTP/dUTP based kit (Cat. No. G476) provide different combinations of deoxyribonucleotides, tailored to the end user's needs and applications.

This kit is provided as individual enzymes to meet the customer's needs and to provide maximum efficiency and flexibility in the RNA sample preparation. The double stranded cDNA end product can subsequently be converted to blunt ended DNA fragments using **abm**'s DNA End Repair Kit (Cat. No. G477), followed by **abm**'s dA Tailing Kit (Cat. No. E009) to generate DNA suitable for whole genome sequencing.

Unit Definition

One unit is defined as the amount of enzyme required to incorporate 1 nmol of deoxynucleotide into acid-precipitable material in 10 minutes at 37°C using Poly(A) and Oligo(dT) as template and primer, respectively.

Applications

1. RNA-Seq Library Construction
2. Downstream double-stranded blunt-end cDNA synthesis for cloning
3. Downstream double-stranded cDNA library construction

Reaction Buffer Components

20 mM Tris-HCl (pH 7.5), 12 mM $(NH_4)_2SO_4$, 10 mM $MgCl_2$, 0.16 mM β-NAD.

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Storage Condition

Store all components at -20°C. All components are stable for 1 year from the date of shipping when stored and handled properly. Avoid repeated freeze-thaw cycles to retain maximum performance.

Protocol

For best results, we recommend the use of **abm**'s **EasyScript™ cDNA Synthesis Kit** (Cat. No. G233) for preparation of your first strand cDNA.

1. Prepare the following reaction mixture on ice:

Components	Volume	Final Concentration
First Strand cDNA (RT products)	Variable	10 ng - 2 µg/rxn
E. coli DNA Ligase (10 U/µl) *E. coli* DNA Polymerase I (10 U/µl) RNase H *E. coli* (5 U/µl)	4 µl 4 µl 4 µl	40 U 40 U 20 U
dNTP Mixture (10 mM each) or dNTP/dUTP Mixture (10 mM each)	1 µl	200 µM
10X Second Strand cDNA Synthesis Buffer	5 µl	1X
Nuclease-free H_2O	Up to 50 µl	-

2. Collect all components by a brief centrifugation. Incubate the reaction at 16°C for 2.5 hours.

3. Chill on ice. The newly generated double-stranded cDNA is ready for immediate downstream applications, or for long-term storage at -20°C.

4. The quantity and size distribution of the synthesized products can be visualized by agarose gel electrophoresis with ethidium bromide or SafeView™ (Cat No. G108) staining.

Troubleshooting

Problem	Possible Cause	Possible Solution
Low yield	Incorrect reaction preparation	Check reaction components and use only the reagents provided
	Incorrect temperature	Incubate reaction at 16°C to prevent spurious synthesis by *E. coli* DNA Polymerase
	Low quality of RNA into the first strand cDNA synthesis	Assess the integrity of RNA prior to cDNA synthesis