



*Enabling Discovery in Life Science®*

# **BioArray™ cDNA Synthesis Kit**

**Instruction Manual**

**Cat. No. ENZ-42406-10**

10 Reactions

***For research use only.***

Rev. 1.0 May 2010

## **Notice to Purchaser**

This product is manufactured and sold by ENZO LIFE SCIENCES, INC. for research use only by the end-user in the research market and is not intended for diagnostic or therapeutic use. Purchase does not include any right or license to use, develop or otherwise exploit this product commercially. Any commercial use, development or exploitation of this product or development using this product without the express prior written authorization of ENZO LIFE SCIENCES, INC. is strictly prohibited.

## **Limited Warranty**

This product is offered under a limited warranty. The product is guaranteed to meet appropriate specifications described in the package insert at the time of shipment. Enzo Life Sciences' sole obligation is to replace the product to the extent of the purchase price. All claims must be made to Enzo Life Sciences, Inc. within five (5) days of receipt of order.

## **Trademarks and Patents**

Enzo and BioArray are trademarks of Enzo Life Sciences, Inc. Several of Enzo's products and product applications are covered by US and foreign patents and patents pending.

# Contents

<b>I. Introduction .....</b>	<b>1</b>
<b>II. Reagents Provided and Storage.....</b>	<b>1</b>
<b>III. Additional Materials Required .....</b>	<b>2</b>
<b>IV. Methods and Procedures .....</b>	<b>2</b>
A. FIRST-STRAND SYNTHESIS .....	3
B. SECOND-STRAND SYNTHESIS .....	4
C. cDNA PURIFICATION USING ENZO No. ENZ-42407 .....	5
<b>V. Appendix—RECOMMENDATIONS FOR     RNASE-FREE TECHNIQUE .....</b>	<b>6</b>
<b>VI. References .....</b>	<b>6</b>



## I. Introduction

The **BioArray™ cDNA Synthesis Kit** provides an optimized protocol and reagents for generation of double stranded cDNA from total cellular samples using a T7-oligo-dT primer system. This system can be used as a first step towards generation of amplified labeled RNA, or it can be used for construction of cDNA libraries.

The cDNA synthesis is performed in two steps. In the first step, the total cellular or tissue derived RNA is primed with T7-oligo-dT and extended using reverse transcriptase at 42°C. In the second step, the primed RNA is converted to double stranded cDNA using RNase H to generate 3'-OH ends suitable for fill-in and extension by DNA Polymerase I. The double stranded cDNA thus generated is a faithful representation of the initial mRNA pool.

## II. Reagents Provided and Storage

Upon receipt, store the **Control RNA (CR)** at -80°C. Store all other kit components at -20°C in a non-frost-free freezer. Water can be left at room temperature after first thawing.

<b>Reagents</b>	<b>Min. Vol. Supplied</b>	<b>Vial ID</b>
dNTP Mix	44 µL	<b>dN</b>
Promoter Primer	11 µL	<b>P</b>
First Strand Buffer	22 µL	<b>FB</b>
DTT	22 µL	<b>D</b>
Reverse Transcriptase	11 µL	<b>RT</b>
RNase Inhibitor	14 µL	<b>I</b>
DNA Polymerase	55 µL	<b>DP</b>
RNase H	11 µL	<b>RH</b>
Second Strand Buffer	170 µL	<b>SB</b>
Nuclease-free Water	1.5 mL	<b>W</b>
Control RNA (100 µg/mL)	10 µL	<b>CR</b>

### III. Additional Materials Required

#### Equipment

- Thermal cycler with heated lid or heating blocks with heated lid
- Cold block (4°C).
- Microcentrifuge
- Orbital shaker for 96-well plates (optional)
- UV Spectrophotometer (optional)
- Bioanalyzer (Agilent, optional)

#### Materials

- Isolated and purified RNA
- 0.2 ml, 0.6 ml and 1.5 ml microcentrifuge tubes
- Sterile, aerosol barrier, nuclease-free pipette tips
- RNase decontamination solution
- Molecular Biology Grade Ethanol (100%)
- cDNA Purification Kit (Enzo Cat. No. ENZ-42407)

### IV. Methods and Procedures

**NOTE:** PLEASE READ THE ENTIRE PROCEDURE BEFORE STARTING. Upon thawing of solutions, gently hand-mix or vortex the reagents prior to use to ensure a homogenous solution. See Appendix for guidelines for RNA preparation.

cDNA synthesis involves two major steps: (1) first strand cDNA synthesis, and (2) second strand cDNA synthesis. The entire procedure may be performed in a thermal cycler (Table 2). Alternatively, heating blocks with heated lids, water baths, or hybridization ovens set at the prescribed temperatures can be used.

Process	°C	Time
Denaturation	70°	10 minutes
	42°	3 minutes
	42°	Pause
Reverse Transcription	42°	120 minutes
	4°	Pause
Second Strand Synthesis	16°	120 minutes
	4°	Pause

For the sake of consistency of common reaction components, we recommend making master mixes for each step of the procedure when performing more than one reaction. The reaction tables show exact reactant volumes per reaction. While making a master mix, include 10% extra volume of each common reaction component in the master mix to make up for losses in distributing into each reaction vial.

## A. FIRST STRAND SYNTHESIS

<b>Component</b>	<b>Vial ID</b>	<b>Vol. per Reaction</b>
First Strand Buffer	<b>FB</b>	2.0 $\mu$ L
DTT	<b>D</b>	2.0 $\mu$ L
dNTP Mix	<b>dN</b>	1.0 $\mu$ L
Reverse Transcriptase	<b>RT</b>	1.0 $\mu$ L
RNase Inhibitor	<b>I</b>	1.0 $\mu$ L
<b>Total</b>		<b>7.0 <math>\mu</math>L</b>

1. Thaw vials **dN**, **P**, **FB**, **D** and **W**. Gently mix, centrifuge (~5 sec) and place on ice until needed. Briefly centrifuge all tubes containing enzymes immediately prior to use.
2. Mix total RNA sample (250 ng - 5000 ng per reaction) with 1  $\mu$ L **Promoter Primer (P)**.
3. Add an appropriate volume of **Nuclease-free Water (W)** to the RNA/Primer mix for a total volume of **13  $\mu$ L**.
4. Incubate for 10 minutes at 70°C to denature. **Do not place** the RNA/Primer mix on ice. Move immediately to 42°C.
5. While denaturing, prepare the First Strand Master Mix by combining the reagents (Table 3) in a sterile, nuclease-free microcentrifuge tube.
6. Gently mix by pipetting up and down several times, centrifuge and place the First Strand Master Mix on ice.
7. Incubate separately both the RNA/Primer mix (from step 4) and the First Strand Master Mix (from step 5) for 3 minutes at 42°C.
8. After 3 minutes at 42°C, add **7  $\mu$ L** of First Strand Master Mix to the RNA/Primer mix and mix by pipetting up and down several times.
9. Incubate for 2 hours at 42°C.
10. Place the tubes on ice.

## B. SECOND STRAND cDNA SYNTHESIS

<b>Component</b>	<b>Vial ID</b>	<b>Vol. per Reaction</b>
Nuclease-free Water	<b>W</b>	106 $\mu$ L
dNTP Mix	<b>dN</b>	3 $\mu$ L
Second Strand Buffer	<b>SB</b>	15 $\mu$ L
DNA Polymerase	<b>DP</b>	5 $\mu$ L
RNaseH	<b>RH</b>	1 $\mu$ L
<b>Total</b>		<b>130 <math>\mu</math>L</b>

1. Thaw vials **dN**, **SB** and **W**, mix, briefly centrifuge and keep on ice.
2. Shortly before the completion of the first strand synthesis reaction, prepare the Second Strand Master Mix (Table 4) in a sterile, nuclease-free microcentrifuge tube. Mix gently by pipetting up and down several times, centrifuge and place on ice.
3. Add **130  $\mu$ L** of the Second Strand Master Mix to the first strand reaction on ice, and mix gently by pipetting up and down several times. Centrifuge briefly to collect the contents.
4. Incubate at 16°C for 2 hours.
5. Place the tubes on ice.
6. Immediately proceed to purification of the double-stranded cDNA template.

### C. cDNA PURIFICATION USING ENZO No. ENZ-42407

**NOTE:** *All centrifugation steps are at 10,000 x g.*

1. Perform the following steps prior to beginning cDNA purification.
  - a. **Make a 1X Wash Solution** by adding 4.8 mL 100% Ethanol to the 1.2 mL of **(WS)** .
  - b. Pre-equilibrate each cDNA purification column with 30  $\mu$ L **DNA Binding Buffer (BB)** for 5 minutes. **Do not centrifuge.**
2. Add 375  $\mu$ L of **DNA Binding Buffer (BB)** to each double-stranded cDNA sample and mix gently.
3. Add cDNA samples in binding buffer to the pre-equilibrated cDNA purification columns and centrifuge for 1 minute. Discard the flow-through.
4. Add 500  $\mu$ L of **1X Wash Solution**, centrifuge for 1 minute, discard the flow-through and centrifuge for an additional minute to remove trace liquid and transfer to an elution tube.
5. Add 17.5  $\mu$ L of pre-warmed (70°C) nuclease-free water, incubate for 1 minute at room temperature and centrifuge for an additional minute to elute cDNA. Repeat this entire step for a total sample volume of 35  $\mu$ L.
6. Purified cDNA can be stored at -20°C for up to 3 days prior to use in an application.

## V. Appendix

### RNA Preparation Guidelines

1. RNA purity and integrity affect both the quantity and quality of the resulting cDNA .
2. Starting with total cellular RNA minimizes loss of low abundance transcripts during mRNA isolation.
3. Most commercially available RNA isolation reagents are compatible with the cDNA Synthesis Kit. However, the Qiagen poly-A carrier RNA is not recommended.
4. Total RNA samples should be in water or buffer, free of protein, DNA, cellular material, organic solvents, salts and other RNA isolation reagents.
5. RNA purity can be assessed via a spectrophotometer with acceptable  $A_{260}/A_{280}$  ratios between 1.8 and 2.1.
6. RNA integrity can be evaluated by gel or by capillary electrophoresis.
7. Both the input RNA and amplified aRNA should be used immediately after purification or stored at  $\leq -70^{\circ}\text{C}$  until use. Results may vary for samples subject to multiple freeze-thaw events.

## VI. References

1. Van Gelder *et al.* PNAS **87**(1990)1663-7. Van Gelder *et al.* PNAS **87**(1990)





[www.enzolifesciences.com](http://www.enzolifesciences.com)

*Enabling Discovery in Life Science®*

**NORTH/SOUTH AMERICA**

**ENZO LIFE SCIENCES INTERNATIONAL, INC.**

5120 Butler Pike  
Plymouth Meeting, PA 19462-1202

USA

T 1-800-942-0430/(610) 941-0430

F (610) 941-9252

E [info-usa@enzolifesciences.com](mailto:info-usa@enzolifesciences.com)

**SWITZERLAND & REST OF EUROPE**

**ENZO LIFE SCIENCES AG**

Industriestrasse 17, Postfach

CH-4415 Lausen

Switzerland

T +41/0 61 926 89 89

F +41/0 61 926 89 79

E [info-ch@enzolifesciences.com](mailto:info-ch@enzolifesciences.com)

[www.enzolifesciences.com](http://www.enzolifesciences.com)

**GERMANY**

**ENZO LIFE SCIENCES GMBH**

Marie-Curie-Strasse 8

DE-79539 Lörrach

Germany

T +49/0 7621 5500 526

Toll Free 0800 664 9518

F +49/0 7621 5500 527

E [info-de@enzolifesciences.com](mailto:info-de@enzolifesciences.com)

[www.enzolifesciences.com](http://www.enzolifesciences.com)

**BENELUX**

**ENZO LIFE SCIENCES BVBA**

Melkerijweg 3

BE-2240 Zandhoven

Belgium

T +32/0 3 466 04 20

F +32/0 3 466 04 29

E [info-be@enzolifesciences.com](mailto:info-be@enzolifesciences.com)

[www.enzolifesciences.com](http://www.enzolifesciences.com)

**UK & IRELAND**

**ENZO LIFE SCIENCES (UK) LTD.**

Palatine House

Matford Court

Exeter EX2 8NL

UK

T 0845 601 1488 (UK customers)

T +44/0 1392 825900 (from overseas)

F +44/0 1392 825910

E [info-uk@enzolifesciences.com](mailto:info-uk@enzolifesciences.com)

[www.enzolifesciences.com](http://www.enzolifesciences.com)

[www.enzolifesciences.com](http://www.enzolifesciences.com)

**FRANCE**

**ENZO LIFE SCIENCES**

c/o Covalab s.a.s.

13, Avenue Albert Einstein

FR -69100 Villeurbanne

France

T +33 472 440 655

F +33 437 484 239

E [info-fr@enzolifesciences.com](mailto:info-fr@enzolifesciences.com)

[www.enzolifesciences.com](http://www.enzolifesciences.com)

incorporating



assay designs®

Stressgen®