コスモ・バイオ株式会社

BioArray™ HighYield™ RNA Transcript Labeling Kit (T7)

Cat. No. 42655-10	1 x 10 labeling reactions
Cat. No. 42655-20	2 x 10 labeling reactions
Cat. No. 42655-20A	1 x 20 labeling reactions
Cat. No. 42655-40	1 x 40 labeling reactions
Cat. No. 42655-100	1 x 100 labeling reactions

For Research Use Only

INTRODUCTION

The ENZO ***BioArray***™ ***HighYield***™ **RNA Transcript Labeling Kit (T7)** has been developed for the production of large amounts of hybridizable biotin-labeled RNA targets by *in vitro* transcription from bacteriophage T7 RNA polymerase promoters. Using T7 RNA polymerase and biotin-labeled nucleotides, large amounts of single stranded nonradioactive RNA molecules can be produced *in vitro*. Because of the nature of transcription reactions, many RNA copies of the template DNA are produced. Because RNA-DNA hybrids have a higher melting temperature than corresponding DNA-DNA hybrids, single-stranded RNA targets offer higher target avidity and greater sensitivity than DNA probes. RNA targets offer selectivity unavailable with DNA targets–being single stranded, they are strand-specific and hybridize more effectively to probes because the target population does not self-hybridize.

The ENZO ***BioArray***™ ***HighYield***™ **RNA Transcript Labeling Kit (T7)** utilizes two different labeled nucleotides, Biotin-CTP and Biotin-UTP, to label the RNA. The use of two different nucleotides enables more uniform and efficient labeling, independent of the nucleotide composition of the RNA produced. The use of a natural nucleotide base derivative enables more efficient incorporation.

RNA transcripts that are labeled with biotin-modified ribonucleotides are used effectively in nucleic acid array assays. The biotin-labeled RNA targets that are hybridized to arrays of DNA probes can be detected by a reporter molecule linked to streptavidin, avidin or anti-biotin antibody. Such a complex can be detected directly, *e.g.*, by excitation of a fluorophore conjugated to streptavidin, or indirectly, *e.g.*, using an enzyme conjugate that can produce an insoluble, colored precipitate.

The ENZO ***BioArray***™ ***HighYield***™ **RNA Transcript Labeling Kit (T7)** has been formulated and optimized for use with nucleic acid array assays. The kit is available in various sizes (see Table 1). Each reaction has been formulated for cDNA made from 1 to 5 µg total RNA or 0.5 to 2 µg mRNA. The T7 promoter containing cDNA can be synthesized using the **ENZO BioArray RNA Amplification Kit** (42401), or any of the other methods currently available.

KIT SIZE OPTIONS AND REAGENTS PROVIDED

Table 1

Reagents	Catalog No. / Package Size				
	42655-10 10 reactions	42655-20 20 reactions	42655-20A 20 reactions	42655-40 40 reactions	42655-100 100 reactions
Vial 1 10X HY Reaction Buffer	1 x 40 µL	2 x 40 µL	1 x 80 µL	1 x 160 µL	1 x 400 µL
Vial 2 10X Biotin-Labeled Ribonucleotides	1 x 40 µL	2 x 40 µL	1 x 80 µL	1 x 160 µL	1 x 400 µL
Vial 3 10X DTT	1 x 40 µL	2 x 40 µL	1 x 80 µL	1 x 160 µL	1 x 400 µL
Vial 4 10X RNase Inhibitor Mix	1 x 40 µL	2 x 40 µL	1 x 80 µL	1 x 160 µL	1 x 400 µL
Vial 5 20X T7 RNA Polymerase	1 x 20 µL	2 x 20 µL	1 x 40 µL	1 x 80 µL	1 x 200 µL

EQUIPMENT AND REAGENTS REQUIRED BUT NOT PROVIDED

- water bath, oven, thermocycler, or heating block set to 37°C
- nuclease-free sterile deionized water
- template DNA containing T7 promoter

STORAGE

Store all reagents at -20°C, in a freezer that is not self-defrosting.

RNA TRANSCRIPT LABELING PROCEDURES

A. Template Preparation

The quality and purity of input RNA and resulting cDNA determine the yield of biotin-labeled RNA.

- cDNA templates should be cleaned up using phenol:chloroform followed by ethanol precipitation. For ethanol precipitation, add ammonium acetate to 2.5M, then add 2.5 volumes of absolute ethanol (-20°C). Immediately centrifuge (≥12,000 x g) at room temperature for 20 minutes. Wash twice with 70% or 80% cold ethanol. After precipitation, spin briefly and aspirate any residual ethanol. Air dry the pellet. Alternatively, cDNA templates can be cleaned with an appropriate DNA purification kit.
- For control plasmid templates, linearize the plasmid DNA using appropriate restriction enzyme digestion. Restriction enzymes that leave a 3' overhang should be avoided because T7 RNA polymerase may transcribe these in a promoter independent manner. Template DNA should be purified before adding to the reaction.
- Use only Nuclease-free water, buffers and pipette tips.

B. RNA Transcript Labeling Reaction

Depending on the starting amount of input RNA, the following values are the recommended amounts of material to use in the HighYield transcript labeling reaction.

Table 2

Starting Material	% cDNA to use in IVT
Total RNA	
up to 100 ng (2 or more rounds of amplification)	100%
1 – 8 µg (1 round amplification)	100%
8-15 µg (1 round amplification)	50%
mRNA	
0.5 – 2 µg	100%
2-4 µg	50%

1. Add reaction components to nuclease-free microfuge tubes.

   Prior to use, spin all components briefly to collect the reagent at the bottom of the tube.

2. Make additions in the order indicated in Table 3.

   Keep reactions at room temperature while additions are made. Spermidine in the reaction buffer may cause precipitation of DNA on ice.

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Table 3

Reagent	Volume
Template DNA	variable (see Table 2)
Distilled or deionized water	variable (to give a final reaction volume of 40 μL)
10X HY Reaction Buffer (Vial 1)	4 μL
10X Biotin-Labeled Ribonucleotides (Vial 2)	4 μL
10X DTT (Vial 3)	4 μL
10X RNase Inhibitor Mix (Vial 4)	4 μL
20X T7 RNA Polymerase (Vial 5)	2 μL
Total Volume	**40 μL**

3. Carefully mix the reagents and collect the mixture in the bottom of the tube by brief (5 second) microcentrifugation.

A master mix of all components except template can be made if multiple reactions are to be performed at the same time.

4. Immediately place the tube in a 37°C water bath, hybridization oven or thermocycler in which the heated lid parallels the block temperature. Incubate overnight (16 hours). If the starting material was greater than 3 µg total RNA, a 4 to 5 hour incubation should be sufficient. If condensation forms, flick material to bottom of tube.
5. Larger amounts of products can be produced by scaling up all components and volumes. In most scale-up syntheses, the amount of DNA template can be reduced to 20% to 50% of scaled up amount.
6. Store labeled RNA at -70°C or less if not purifying immediately.

TROUBLESHOOTING

Observation	Suggestions/Comments
Precipitate in the reaction buffer or DTT	After many freeze-thaw cycles, a precipitate may form. If the precipitate does not solubilize after gentle mixing, do not use.
Low yield	The most likely cause of low yield in a transcription reaction is poor quality or impure template. Carry over of phenol will inhibit the reaction. To remove phenol, wash the template twice with 70% or 80% ethanol.
	The presence of excess T7 promoter-containing primers during synthesis of cDNA template can also decrease yield. Following synthesis of the cDNA template the primers can be removed by precipitating the cDNA with 2.5M ammonium acetate and 2.5 volumes of absolute ethanol. The precipitate should be spun immediately at room temperature for 20 minutes. If other salts are used or if the sample is frozen the primers may also precipitate resulting in their incomplete removal. If interference by excess primers persists, the starting concentration of primers can be reduced in cDNA synthesis reaction. This is recommended when starting with reduced amounts of RNA.
	Some cDNA synthesis reactions may produce cDNA that has been primed with RNA instead of the T7 promoter-containing oligo primer. This is more likely to occur when starting with low quality RNA. The RNA-primed cDNA contains no T7 promoter sequence and thus will not be transcribed.

PURIFICATION OF LABELED RNA TRANSCRIPTS

We recommend RNeasy mini columns from QIAGEN for purification of labeled RNA, with the following modifications for biotinylated RNA: adjust the sample volume to 80 µl with nuclease-free water. Add 280 µl Buffer RLT (ß-mercaptoethanol is not needed, and may cause background on the array). Mix thoroughly. Then add 340 µl ethanol (96-100%) to the diluted RNA and mix thoroughly by pipetting up and down. Do not centrifuge. Proceed to the column as described by Qiagen. When eluting the RNA from the column, allow the water eluent to remain layered on the column membrane for two minutes before spinning.

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Technical Support

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Biotin Labeling for Gene Expression Analysis

BioArray™ ***HighYield***™ *RNA Transcript Labeling System*

The Enzo ***BioArray***™ ***HighYield***™ RNA Transcript Labeling System is the gold standard transcript labeling system and is intended for use with Affymetrix® GeneChip® brand eukaryotic expression microarrays. This proprietary labeling chemistry is referenced in thousands of research publications citing the use of GeneChip® expression arrays. Recently, labeling substitutes have appeared in the market tempting researchers to unknowingly compromise the quality of the generated data. It is evident different labeling chemistries generate very different expression profiles, which leads to the question: If the profiles are different, which labeling method produces the higher quality data set?

The Enzo ***BioArray***™ ***HighYield***™ System was compared with a recently marketed labeling substitute. The experiment highlights are described in *Proven Performance*.

Proven Performance

Studies to compare the gold standard Enzo ***HighYield***™ system with the new Affymetrix® IVT labeling kit were conducted by an independent laboratory. A total of four 2 µg samples of RNA (reference total RNA from Stratagene® La Jolla, CA) were used to generate double stranded cDNA by a modified Eberwine protocol.

The double stranded cDNA was pooled and redistributed into two Affymetrix® and two Enzo ***HighYield***™ *in vitro* transcriptions. Each reaction was incubated for 16 hours, after which purified cRNA (15 µg) from each IVT reaction was hybridized onto Affymetrix® HU133 2.0 Plus Arrays using either fluidics script v4 or v5.

The table below summarizes the results generated from the comparison experiment. aRNA yield and size distribution for both Enzo and Affymetrix® methods are statistically equivalent. Evaluation of percent present call (% P call), scale factor, background, GADPH 3'/5' and Actin 3'/5' demonstrates the Enzo ***HighYield***™ system consistently yields higher % P calls for either v4 or v5 fluidics. On average, 2500 additional transcripts are seen as present using the Enzo ***HighYield***™ system as compared with the new Affymetrix® kit.

Table1: Enzo displays higher % P calls

IVT Kit	Affymetrix®				Enzo			
Sample/Script	1/v4	1/v5	2/v4	2/v5	1/v4	1/v5	2/v4	2/v5
% P call	**31.2**	**33.6**	**29.4**	**31.5**	**35.6**	**36.8**	**34.9**	**39.0**
Scale Factor	10.312	12.547	11.758	15.27	7.358	12.156	8.096	12.46
Background	64.66	58.04	68.1	56.43	46.52	34.16	40.67	30.62
GADPH 3'/5'	1.13	1.07	1.08	1.05	0.99	0.97	1.02	1.02
Actin 3'/5'	1.87	1.76	1.81	1.76	1.44	1.71	1.53	1.83

Applications:

+ Affymetrix® GeneChip® Brand Eukaryotic Expression Arrays; Illumina Sentrix® BeadChip Arrays; Combimatrix Expression Arrays; NimbleGen Expression Arrays

Advantages:

+ Decreases experimental variation and standardizes biological data derived from microarrays with the universally accepted ***HighYield***™ system.
+ Maximizes incorporation efficiency and produces strong, clear displays by utilizing 2 biotin-labeled nucleotides and a high quality T7 polymerase.
+ Compatible with both new and existing Affymetrix® arrays.
+ Maintains the value of your legacy data by the continued use of Enzo, the gold standard enabling GeneChip® visualization.
+ Facilitates workflow with a flexible 4-16 hour transcription time and reagents supplied in a convenient, ready to-use format.
+ Enables correlation of results from experiment to experiment, project to project and lab to lab.

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Summary

The results generated by the independent lab in the comparison study are conclusive that:

- Enzo calls a significantly greater number of transcripts as present.
- Microarrays used with Enzo have much lower background.
- The 3'/5' ratios with Enzo are consistently lower.
- Enzo data is enhanced appreciably by recent updates to the fluidics washing steps in the GeneChip® protocol.
- Enzo data is considered a reproducible standard where the value of your legacy data is maintained by the continued use of the Enzo Technology.

The ***BioArray™ HighYield™*** RNA Transcript Labeling System addresses the critical step for efficient labeling of RNA for microarrays. Yields of 100 µg or greater are produced by a T7 RNA Polymerase catalyzed *in vitro* transcription in the presence of both biotin labeled UTP and CTP. The use of two nucleotides yields a high incorporation efficiency in RNA consistently across all tissue types.

Together with a time saving protocol, the Enzo ***BioArray™ HighYield™*** system is the method of choice for improving GeneChip® visualization. Enzo's labeling technology maximizes the value of your biological samples as well as your microarray experiments and databases.

For more information or to place an order, contact your Enzo Life Sciences' Alliance Manager at 1.800.221.7705 or 1.631.694.7070 or visit our web site at ***www.enzolifesciences.com****.*

Ordering Information >>

Catalog Number	Description	Quantity
42655-10	***BioArray™ HighYield™*** RNA Transcript Labeling System (T7)	10 labeling reactions
42655-20A		20 labeling reactions
42655-40		40 labeling reactions
42655-100		100 labeling reactions

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