

BioArray HighYield[®] RNA Transcript Labeling Kit (T7)

Catalog #: [ENZ-42655](#)

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

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Please read
entire booklet
before
proceeding with
the assay.



Carefully note
the handling
and storage
conditions of
each kit
component.



Please contact
Enzo Life
Sciences
Technical
Support if
necessary.

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INTRODUCTION

Enzo Life Sciences' 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. Because of the nature of transcription reactions, many RNA copies of the template DNA are produced. The kit 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 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 5µg total RNA or 0.5 to 2µg mRNA. The T7 promoter containing cDNA can be synthesized using the BioArray[™] cDNA Synthesis Kit (ENZ-42406-10), 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	42655-20A 20 reactions	42655-40 40 reactions	42655-100 100 reactions
Vial 1 10X HY Reaction Buffer	40µl	80µL	160µL	400µL
Vial 2 10X Biotin-Labeled Ribonucleotides	40µL	80µL	160µL	400µL
Vial 3 10X DTT	40µL	80µL	160µL	400µL
Vial 4 10X RNase Inhibitor Mix	40µL	80µL	160µL	400µL
Vial 5 20X T7 RNA Polymerase	20µL	40µL	80µL	200 µL

OTHER MATERIALS NEEDED

- 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

Template Preparation

- 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 ($\geq 12,000 \times g$) 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.

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

- 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.

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 RNA 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 - 8µg (1 round amplification)	100%
8 - 15µg (1 round amplification)	50%
mRNA	
0.5 - 2µg	100%
2 - 4µg	50%

1. Add reaction components to nuclease-free microfuge tubes.
NOTE: 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**.
NOTE: Keep reactions at room temperature while additions are made. Spermidine in the reaction buffer may cause precipitation of DNA on ice.

Table 3

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

3. Carefully mix the reagents and collect the mixture in the bottom of the tube by brief (5 second) microcentrifugation.
NOTE: 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 3μg 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.
6. Store labeled RNA at -70°C or less if not purifying immediately.

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 80µL with nuclease-free water. Add 280µL Buffer RLT (β -mercaptoethanol is not needed, and may cause background on the array). Mix thoroughly. Then add 340µL 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.

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 of or impure template. Carry over of phenol will inhibit the reaction. To remove phenol, wash the template twice with 70% or 80% ethanol after precipitation.
	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.



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