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Latest LSC technology cuts sample and cocktail costs; increases throughput

In the past, liquid scintillation counting used traditional technology to overcome background noise. Now, there's a new, more sensitive technology - TR-LSC or Time-Resolved Liquid Scintillation Counting - that reduces background noise by an additional 30%-40%, and more. This new patented technology is available only in Packard's Tri-Carb® liquid scintillation analyzers.

Originally developed for extremely low level counting, TR-LSC technology has now been applied to a broad range of applications. While these don't always require high sensitivity, additional benefits have been realized. By increasing sensitivity, TR-LSC reduces sample and cocktail consumption while shortening the time required for accurate counts. The benefits? Lower cocktail costs, lower disposal costs, and increased throughput.

How TR-LSC is superior to older technology

Traditional counters are based on two-dimensional pulse analysis: pulse height and pulse counts. They provide a level of sensitivity that's merely adequate for most applications.

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USB introduces RNAzyme™ Tet 1.0 (Ribozyme Tet 1.0) cucu specific: the first commercially-available Ribozyme for sequence-specific cleavage of RNA.

The discovery of ribozymes — novel enzymes composed entirely of RNA that cleave RNA in a sequence-specific manner — represents a tremendous opportunity to expand the scope of RNA studies.

Now USB scientists have produced the first commercially-available ribozyme from a naturally-occurring, self-splicing group I intron of Tetrahymena RNA (1). RNAzyme™ Tet 1.0 (Ribozyme Tet 1.0) cucu specific is one of a group of important ribozymes (sequence-dependent RNA endonucleases), that promise to be powerful tools in experiments involving all aspects of RNA. RNAzyme™ Tet 1.0 (Ribozyme Tet 1.0) cucu specific resembles a DNA restriction endonuclease in that it will cut RNA into discrete segments at a specific nucleotide sequence; in this case, cleaving RNA at sites having the sequence cucu. As with DNA restriction endonucleases, the concept of using a ribozyme as a molecular scissors is simple. However, its potential value lies in the development of imaginative applications which, until now, have been difficult or impossible to achieve.

One primary application will, of course, be in the physical mapping of related RNA species. In addition, consider RNA sequencing, secondary structure analysis and in vitro RNA metabolic studies.

We are excited to take the leading role in the development of this newly-discovered field, and dedicated to fulfilling the promise ribozymes represent as extremely useful tools in basic molecular biological research, as well as in other areas as diverse as oncology, virology, pharmacology and agriculture.

USB RNAzyme™ Tet 1.0 (Ribozyme Tet 1.0) cucu specific is offered in a kit with all the accessory reagents necessary to calibrate the ribozyme activity with any RNA substrate and begin RNA analysis.

As always, look to USB for specific and efficient molecular tools to handle innovative applications in biology.

(Additional information on next page.)

*Patents pending.

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**FIG. 1** Demonstration of the Specificity of Cleavage. RNAzyme™ Tet 1.0 was incubated with seven different oligomeric RNA substrates: lanes 1 and 2, 5'GGGACUCAAAAAA3'; lane 3, 5'GGGACAUUAAAAAA3'; lane 4, 5'GGGACGCCAAAAAA3'; lane 5, 5'GGGACCAAAAAAAA3'; lane 6, 5'GGGACCAACAAAAAAA3'; lane 7, 5'GGGACUAUAAAAAAA3'; lane 8, 5'GGGACGAAAAAAA3'. Lane 1 is a no ribozyme control. The reaction contained 0.10μM substrate ([α-32P] ATP labeled), 0.01μM RNAzyme™ Tet 1.0, 2.5M urea, 0.5M GTP, 20mM MgCl2, 50mM Tris-HCl (pH 7.5). Incubation was for 1 hour at 50°C. Samples from each reaction were electrophoresed on a high percentage acrylamide denaturing gel and the bands of substrate and digestion products were identified by autoradiography. Only the matched substrate in lane 2 was cleaved.

**FIG. 2** RNA Fragments of SV40 and Physical Map. SV40 RNA (610-nt) was glyoxalated and then cleaved with RNAzyme™ Tet 1.0 at 50°C in a reaction containing 1.5M urea. The RNA fragments were detected by two different labeling schemes which together identify the 5'-terminal fragment. The lanes are as follows: lane 1, 32P-labeled RNA transcript uncut; lane 2, unlabeled RNA cut with ribozyme in the presence of [32P]GTP; lane 3, body-labeled RNA cut in the presence of unlabeled GTP; M, glyoxalated Haelll fragments of φX DNA. The fragments were separated by electrophoresis in a 4% acrylamide gel containing 8M urea and the bands were visualized by autoradiography. The physical map shows the locations and sequences of the RNAzyme™ Tet 1.0 cleavage sites in SV40.

The results shown illustrate the site-specific cleavage activity of RNAzyme™ Tet 1.0 on both oligomeric and larger RNA. They confirm the results originally reported for the Tetrahymena group I intron derived ribozymes (1,2). This work provides the basis for the experimenter to generate digestion patterns of larger RNA and to attempt studies involving the detection of different forms of RNA (e.g., alternatively spliced RNA) in complex populations. Studies involving in vitro transformation of purified RNA fragments could also be possible. The development of RNAzyme™ Tet 1.0 to its full potential as a biological tool will depend upon further characterization of its enzymatic activity as well as the development of specific protocols.

The RNAzyme™ Tet 1.0 kit includes sufficient ribozyme and reagents to allow a variety of investigations. The Control Substrate in the kit is provided to allow calibration of the RNAzyme™ Tet 1.0 activity since activity is dependent upon the concentration of GTP, urea, and cleavage sites as well as the ribozyme concentration. Selection of various conditions for cleavage depends upon the fraction of sites needed to be cleaved; the amount of radiolabel needed to be incorporated into the fragments and the degree of sequence specificity desired.

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**PARTIAL LIST OF SPEAKERS FOR HUMAN GENOME I**

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<td>Distincted Research Professor and Acting President, Salk Institute</td>
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<td>Professor of Applied Microbiology, University of Tokyo</td>
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**PARTIAL LIST OF SESSION TOPICS**

- Organization of the Human Genome Project
- State of our Current Knowledge
- Advances in Technology: New Methods for Mapping and Sequencing
- Progress in Interesting Regions of the Human Genome
- The Diversity of the Human Genome in Different Populations

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