New Protein Truncation Test Kit Simplifies Mutation Detection

Protein Truncation Test detects mutations at the protein level

The Protein Truncation Test (PTT) is a mutation-screening method that allows researchers to detect mutations at the protein level rather than the DNA level (1). The PTT detects "nonsense" or "stop" mutations, the most prevalent mutations in several disease-related genes, which prematurely terminate translation and produce a truncated protein unable to function like the normal protein.

The Protein Truncation Test has proved particularly useful in the study of human disease genes. For example, nonsense mutations account for up to 98% of the mutations in the APC (Adenomatous Polyposis Coli) gene associated with an inherited form of colon cancer (2) and up to 86% of the mutations in the BRCA1 gene linked to breast cancer (3). Faster and more convenient than mutation screening by DNA sequencing, the Protein Truncation Test has also successfully detected truncated proteins encoded by genes linked to Duchenne Muscular Dystrophy (1) and Hereditary Non-Polyposis Colon Cancer (4).

New kit provides safety, convenience, and reliability

Boehringer Mannheim’s new Protein Truncation Test, increases the convenience of PTTs with optimized reagent premixes for in vitro transcription and translation reactions following PCR (Figure 1). These reagent premixes improve the reliability of PTTs by minimizing the number of pipetting steps and avoiding the optimization of reaction mixtures. In addition, the translation premix employs biotin as a protein label, which eliminates the safety concerns, disposal hassles, and record keeping required by radioactive PTTs using 35S-methionine.

Detection of the biotinylated translation products in a chemiluminescent reaction (Figure 2) produces results much more quickly than the day-long film exposures required by radioisotopic PTTs. The complete PTT procedure, from PCR product to chemiluminescent detection, takes less than 6 hours.

Each lot of kits is function tested (with the provided control DNA and control primers) in an actual Protein Truncation Test, ensuring success from PCR through translation and detection. In addition, the kit’s convenient biotinylated molecular weight marker facilitates accurate determination of protein size.

The Protein Truncation Test is now available

Order the Protein Truncation Test, non-radioactive, (Cat. No. 1 888 439) from your local Boehringer Mannheim Biochemicals representative. Or, for additional information visit http://biochem.boehringer-mannheim.com on the Internet.

References

This product is sold under licensing arrangements with Roche Molecular Systems and The Perkin-Elmer Corporation. Purchase of this product is accompanied by a license to use it in the Polymerase Chain Reaction (PCR) process in conjunction with an authorized thermal cycler.

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BOEHRINGER
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DNA Methylation, Imprinting, and the Epigenetics of Cancer

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SCIENTIFIC PROGRAM

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Arthur D. Riggs / Duarte, CA

Tumor Suppressor Genes
Stephen B. Baylin / Baltimore, MD
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Susan J. Clark / Sydney, Australia

Methylation Patterns
Timothy Bestor / Columbia, NY
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Samuel H. Speck / St. Louis, MO
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Chromatin Structures
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Alan P. Wolff / Bethesda, MD
Steven Henikoff / Seattle, WA

Mismatch Repair and Methylation
Donald Kohn / Los Angeles, CA
Christoph Lengauer / Baltimore, MD
Jean-Pierre J. Issa / Baltimore, MD

Methylation and Mutation
Joseph Jiricny / Zurich, Switzerland
Gerd P. Pfeifer / Duarte, CA
Peter A. Jones / Los Angeles, CA

Applicants are encouraged to submit abstracts for poster presentation.

Application deadline: September 30, 1997

Information and Application Forms
American Association for Cancer Research
Public Ledger Building, Suite 826
150 South Independence Mall West
Philadelphia, PA 19106-3483
215-440-9300 215-440-9313 (FAX)
aacr@aacr.org (E-mail)
http://www.aacr.org
**PCR ELISA Kits Simplify PCR Analysis and Quantification**

Boehringer Mannheim kits provide an ideal system for genetic analysis and quantitative PCR

The PCR ELISA system provides several technical advantages compared to traditional PCR analysis methods. First, the sensitivity of ELISA detection of PCR products is 10–100 fold greater than fluorescent staining on agarose gels. Second, the use of a capture probe provides verification of the authenticity of the PCR reaction product. And third, the use of an ELISA format affords rapid analysis of multiple PCR reactions simultaneously.

### Capture probe technology allows precise characterization of the sequence of PCR products

Many current PCR applications such as HLA genotyping, the analysis of microorganisms in biological samples, and the detection of specific gene mutations require precise verification of PCR product specificity. Because the PCR ELISA system utilizes a sequence-specific capture probe, it can easily be adapted to these protocols by simply varying the nucleotide sequence of the capture probe to correspond to the desired target sequence. Discrimination of point mutations can be readily accomplished by modifying the stringency of the probe hybridization and washings conditions (Figure 2).

### Quantitative PCR assays are simple and affordable

Quantitative PCR studies require the analysis of multiple PCR reactions. Traditional methods for quantitative PCR analysis are laborious or involve the purchase of complicated expensive equipment. Boehringer Mannheim's PCR ELISA system provides a simple affordable alternative. The PCR ELISA system allows multiple samples to be processed in parallel in standard 96-well microtiter plates. Multichannel pipettors and a standard microtiter reader is all that is required to obtain accurate reliable quantitative PCR results. If fewer than 96 wells are required, the microtiter plates can be readily separated into 8-well strips to allow for economical use of the kit components.

### A complete system of PCR ELISA kits is now available

Three kits comprising the PCR ELISA system are available from Boehringer Mannheim. Choose one of two labeling kits, in combination with the PCR ELISA Detection Kit, to fit the needs of your experiments (Table 1). Order the PCR ELISA system from your local Boehringer Mannheim representative or, for more information, visit our internet web site at http://biochem.boehringer-mannheim.com.

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<td>PCR ELISA DIG Labeling®**</td>
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<td>PCR ELISA (DIG Detection)†</td>
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* This product is accompanied by a limited license to use it in the Polymerase Chain Reaction (PCR) process for life science research in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Perkin-Elmer or as purchased, i.e., an authorized thermal cycler.
† This product is sold under licensing arrangements with Roche Molecular Systems and The Perkin-Elmer Corporation.

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- ATM and Cell Cycle Control
- Stress Responses and Oxidative Stress in A-T
- Insights From Animal Models and Their Derivatives
- Neurobiology of A-T
- ATM-related Proteins in Various Organisms
- ATM Alterations in Malignancies
- A-T Carriers and Cancer Predisposition
- The Nijmegen Breakage Syndrome

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- Martin Lavin, PhD - Queensland Medical Research Institute, Australia
- Yosef Shiloh, PhD - Tel Aviv University, Israel

**REGISTRATION FEES**

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*Only full-time employees of government and universities may attend at the reduced rate.

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To request specimens and data, send a short letter outlining the aims of the proposed research to the address shown below. Briefly describe the technical approach and proposed technique(s) that can be applied to paraffin-embedded specimens. Include an estimate of the number of cases needed for the study. The request will be reviewed upon receipt and a more detailed proposal will be requested if the study appears reasonable and the Resource has the requested materials. There are no specific receipt dates and every attempt will be made to evaluate requests expeditiously.

Additional information may be obtained on the Resource web site, which contains a searchable database of cases, at: http://wwwicirc.nci.nih.gov/index/html or from: Sherrill Long, Information Management Services, Inc.

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Transcriptional Control of Proliferation, Differentiation, and Development

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SCIENTIFIC PROGRAM

Keynote Session
Michael G. Rosenfeld / San Diego, CA
Stephen K. Burley / New York, NY
Michael R. Green / Worcester, MA

Transcriptional Mechanisms
Richard A. Young / Cambridge, MA
Joan W. Conaway / Oklahoma City, OK
James L. Manley / New York, NY
Cynthia Wolberger / Baltimore, MD

The Influence of Chromatin Structure on Transcription
Beverly M. Emerson / La Jolla, CA
Alan P. Wolfe / Bethesda, MD

Transcriptional Regulation of the Cell Cycle
David M. Livingston / Boston, MA
Bruce A. Edgar / Seattle, WA
Charles J. Sherr / Memphis, TN
Erlin K. O'Shea / San Francisco, CA

Signal Transduction and Transcription
Gerald R. Crabtree / Stanford, CA
Joan Massague / New York, NY
Hans C. Clevers / Utrecht, The Netherlands

Oncogenic and Anti-Oncogenic Transcription Factors
Carol Prives / New York, NY
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Transcription Control of Differentiation
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Gene Manipulating Strategies
Robb Krumlauf / London, England
Spyros Artavanis-Tsakonas / New Haven, CT
Eric N. Olson / Dallas, TX
Norbert Perrimon / Boston, MA

Applicants are encouraged to submit abstracts for poster presentation.

Application deadline: July 31, 1997

Information and Application Forms
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Public Ledger Building, Suite 816
150 South Independence Mall West
Philadelphia, PA 19106-3483
215-440-9300  215-440-9313 (FAX)
E-mail: aacr@aacr.org
The cover of this issue pays tribute to Norma Wollner, a distinguished clinical scientist, who is Professor of Pediatrics at Cornell University School of Medicine and Attending Pediatrician at Memorial Sloan-Kettering Cancer Center, NY. Born in Sao Paulo, Brazil, she received her early education and medical training at the University of Sao Paolo, where she received her M.D. in 1950. After postdoctoral training in Brazil, she came to the United States in 1958 as a Fellow in Medicine and Pediatric Hematology at what was then called the Memorial Hospital for Cancer and Allied Diseases. After further training in pediatrics at Bellevue Hospital, New York University Medical School, and Cornell University Medical College, she joined the pediatric group at Memorial Hospital in 1966, where she has remained ever since.

Among Dr. Wollner’s major contributions to the field of chemotherapy is the development of novel and highly effective protocols for the treatment of childhood non-Hodgkin’s lymphoma. Based on canine research in the pharmacology laboratories in the Sloan-Kettering Institution, Dr. Wollner demonstrated the feasibility of giving methotrexate intrathecally and developed the protocol called LSA2-L2, a combination of high-dose cyclophosphamide, radiation, and combinations of prednisone, vincristine, daunomycin, and intrathecal methotrexate, to be used in the treatment of childhood non-Hodgkin’s lymphoma. Prior to 1972, only 10% of those with the disease survived, with a median period of six months. By 1976, Dr. Wollner and colleagues reported the achievement of a disease-free survival rate of 76% (Cancer, 37: 123–134, 1976), and this remarkable advance has been closely maintained for over 20 years. Dr. Wollner also developed a protocol for treatment of germ-cell tumors, including ovarian teratomas, which led to 80% survival rates, with a median observation of 40 months.

Another unique accomplishment of Dr. Wollner’s, which exemplifies her devotion to assisting those with childhood cancer, was her development of the concept of the Pediatric Day Hospital. Under the Day Hospital system, children with illnesses are able to undergo their daily treatments at the hospital and return to their homes later the same day, thus allowing them to benefit from the comfort of familiar surroundings during what can be a very traumatic experience. At the Day Hospital, treatment includes such procedures as transfusions, radiation, chemotherapy requiring prolonged hydration, and stem cell harvesting and infusion. Treatment requiring anesthesia is generally performed with a short-acting form, thus allowing the patient to return home within a few hours. This effective and compassionate system greatly eases the burden of patients and their families and is now widely practiced. According to Dr. Wollner, who has been Director of the Pediatric Day Hospital in New York since 1978, at its start in 1969 the Day Hospital patient load was 5 to 10 per day, while today it is up to 75 to 80 patients per day. Throughout the years, the Pediatric Day Hospital has been a national model not only for programs for children with cancer but for those with other serious illnesses as well.

Dr. Wollner’s commitment to the elimination of the terrible consequences of childhood cancer is illustrated by the following statement from Lear’s magazine (p. 26, January 1991): “Before I begin consultations with patients at about 7:30 each morning, I spend an hour answering letters, filling out forms, and checking laboratory reports. I try to get the administrative business out of the way as early as I can, so that when the first young outpatient arrives at the Day Hospital, the real challenges of the work—and the real joys—can begin.”

Dr. Wollner is an author or co-author of over 60 articles, reviews, and book chapters and is certified as a member of the American Board of Pediatrics. She is also a member of the American Society of Clinical Oncology, the American Radium Society, the International Society of Pediatric Oncology, and the Society for the Study of Blood. She has received many honors, including the Blue Cross Medal presented by the President of Brazil, the Distinguished Oncologist Award from the Dayton Oncology Society, and a citation from the borough of Manhattan for an outstanding medical career and for philanthropic activities in founding the Brazilian Children’s Fund.

We are indebted to Daniel S. Martin for his assistance in providing information on Dr. Wollner’s career.

Sidney Weinhouse