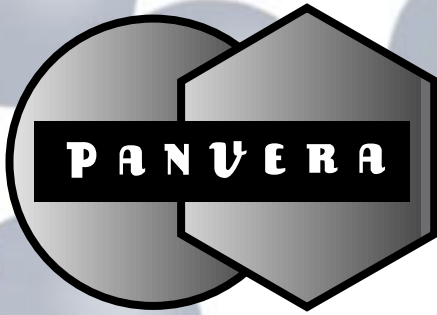


Postings

Spring 1998

Published quarterly by PanVera Corporation, Madison, Wisconsin



“We especially need imagination in science. It is not all mathematics, nor all logic, but it is somewhat beauty and poetry.”

Maria Mitchell

Label IT™ Non-Radioactive Labeling Reagents: Covalent, One-Step Nucleic Acid Labeling

*Paul M. Slattum, Lisa J. Hanson and James E. Hagstrom
Mirus Corporation*

INTRODUCTION

Efficient, simple, and reliable non-radioactive DNA labeling is essential to a wide variety of molecular and cell biology applications. Both enzyme-mediated and direct labeling protocols have been developed to attach tags, such as the fluorescent compounds fluorescein and rhodamine, to DNA. While these labeling methods have allowed non-radioactive detection systems to approach or even surpass radioactive methods in terms of sensitivity, they currently possess significant disadvantages. Enzymatic DNA labeling systems require a number of reagents including both unlabeled and labeled nucleotide precursors, primers, and/or enzymes to facilitate DNA synthesis. Labeling efficiency is not easily controlled. The two most common labeling reactions (nick translation and random priming) do not produce a labeled probe that is the same size as the starting DNA. Conventional direct labeling methods are limited by reduced sensitivity due to a low efficiency of labeling. The protocols are often laborious, involve harsh reaction conditions, and produce variable results.

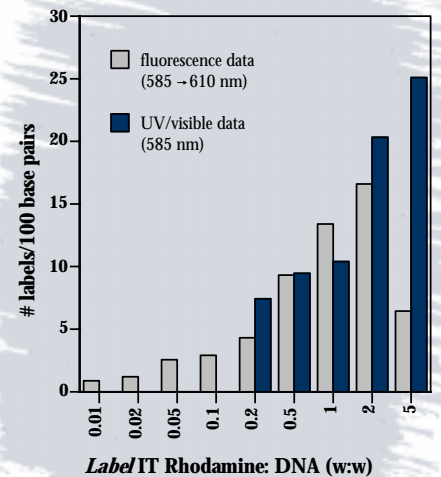


Figure 1

Labeling efficiency with Label IT Rhodamine.

Mirus Corporation has developed a series of *LabelIT* Reagents consisting of modified non-radioactive reporter molecules (rhodamine, fluorescein, digoxin, or biotin) that covalently attach to single- and double-stranded DNA, and RNA. The labeling procedure consists of a 0.5-1.0 hr incubation at 37°C, followed by spin-column purification, resulting in efficient, non-destructive labeling of any polynucleotide. When labeling double-stranded nucleic acids, the label attaches to guanine residues. The site of modification is not involved in base pairing, making this method an excellent choice for hybridization experiments.

RESULTS

High-efficiency fluorescent labeling

To quantitatively determine the level of labeling provided by *LabelIT* Reagents, we

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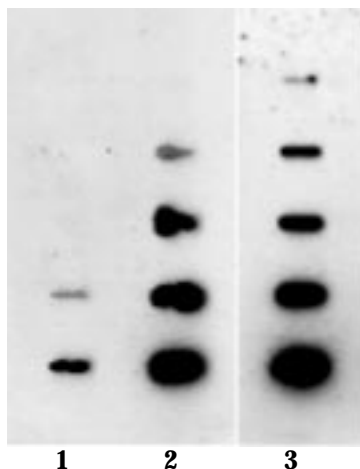


Figure 2

Chemiluminescent detection of digoxin and digoxigenin labeled DNA. Lane 1, random prime labeling (digoxigenin) of a 1.6 kb linear DNA using a kit from company B; lane 2, a 1.6 kb linear DNA fragment labeled with Label IT Digoxin; lane 3, a 5.8 kb supercoiled plasmid labeled with Label IT Digoxin.

Label IT™ Labeling Reagents

(continued from previous page)

labeled plasmid DNA at increasing ratios of *LabelIT* Rhodamine reagent to DNA. We measured the extent of labeling using two different criteria. As seen in Figure 1, there is a linear increase in labeling efficiency as the ratio of *LabelIT* Reagent to DNA is increased. It is possible to achieve very high labeling efficiencies (1 label per 4 base pairs) by increasing the reagent to DNA ratio. It is readily apparent that, at the highest labeling ratios, there is a large discrepancy between the calculated labeling efficiencies using the two different quantitation methods. As labeling density approaches and exceeds 1 label per 10 base pairs, fluorescence quenching of the adjacent rhodamine molecules becomes significant. It is important to keep this observation in mind when designing experiments and calculating the optimal amount of fluorescent modification to use for a particular application. Absorbance values (585 nm) are not similarly affected and thus represent the actual level of modification of the nucleic acid. At the lowest ratios of labeling (<0.1:1) only rhodamine fluorescence values are shown because the sensitivity of absorbance (585 nm) detection is significantly lower than that of fluorescence detection.

High-efficiency digoxin labeling for chemiluminescent detection

To determine the detection limits for digoxin-labeled DNA, we labeled linear

and supercoiled DNA with *LabelIT* Digoxin, and a random-primed digoxigenin labeling kit from another manufacturer. The labeled DNA was then applied to a nylon membrane in varying amounts and detected with an anti-digoxigenin HRP conjugate, using a chemiluminescent substrate. Figure 2 shows that sub-picogram levels of DNA could be detected by this method. Further, the *LabelIT* method provided greater sensitivity than the random-primed labeling kit.

Labeling is covalent and non-destructive

We examined the integrity of DNA labeled with *LabelIT* Rhodamine, using three DNA fragments. The DNA was labeled and purified, and both the rhodamine-labeled and unlabeled fragments were analyzed by agarose gel electrophoresis (Figure 3). The rhodamine-labeled DNA fragments were visualized directly at 302 nm, indicating a covalent linkage of the label to the DNA. The unlabeled DNA fragments were stained with ethidium bromide. Both the rhodamine-labeled fragments and the corresponding unlabeled fragments migrated similarly in the gel, indicating that the DNA fragments remained intact after labeling.

CONCLUSIONS

LabelIT Reagents can be used to label DNA efficiently, simply, and non-destructively. Using *LabelIT* Rhodamine, we

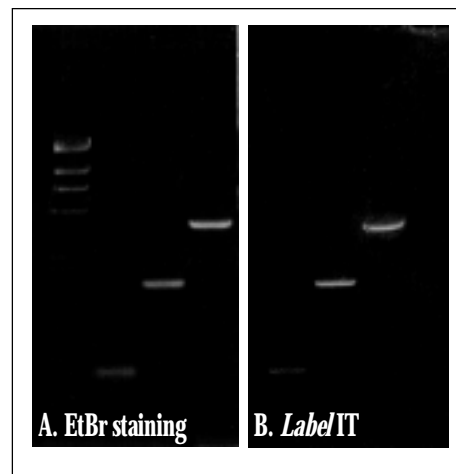


Figure 3

Agarose gel electrophoresis of unlabeled (A) and rhodamine-labeled DNA (B).

demonstrate that the labeling reaction is covalent and non-destructive. We observed sensitive, sub-picogram detection levels of DNA labeled with *LabelIT* Digoxin. The versatility, high efficiency, and simplicity of the *LabelIT* Reagents make them an ideal choice for a variety of non-radioactive labeling applications.

Circle response number 100



Paul M. Slattum

MATERIALS AND METHODS

Determination of labeling efficiency

Plasmid DNA (pCILuc) was reacted with *LabelIT* Rhodamine reagent at the ratios (w:w) of labeling reagent to DNA indicated in Figure 1. The labeled DNA was purified using ethanol precipitation and diluted to 0.5 ml with distilled water. The extent of labeling was determined by using two criteria: fluorescence intensity of the attached rhodamine (excitation at 585 nm, emission at 610 nm), and visible absorbance at 585 nm. The values obtained were then divided by the absorbance at 260 nm to correct for slight variations in the absolute amount of DNA present.

Detection sensitivity

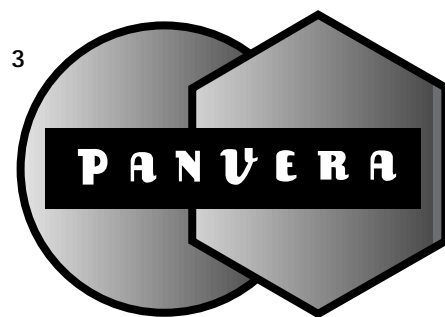
A 1.6 kb linear DNA fragment and supercoiled plasmid DNA were labeled with *LabelIT* Digoxin according to the standard labeling protocol using a 1:1 (w:w) reagent:DNA ratio. The linear DNA was also labeled with a commercially available random-prime digoxigenin labeling kit, according to the manufacturer's instructions. After spin column purification, digoxin-labeled DNA was alkaline-denatured, neutralized, and applied to a nylon membrane (MAGNACHARGE, Micron Separations Inc.) using a slot blot apparatus. Membrane slots were washed 1X with buffer (20mM MOPS, pH 7.5), air dried, and baked for 2 hours at 80°C. Digoxin-labeled DNA on the membrane was reacted with anti-digoxin-HRP (Pierce; Rockford, IL) and detected using Super Signal chemiluminescent substrate (Pierce; Rockford, IL) according to manufacturer's recommendations. Figure 2 is an

example of a 10 minute exposure using Kodak Biomax ML film (Eastman Kodak; Rochester, NY). Similar results were obtained with an anti-digoxigenin antibody (data not shown).

Integrity of labeling

Three linear DNA fragments (3468, 1665 and 565 bp) were labeled using *LabelIT* Rhodamine following the standard protocol with a 0.2:1 (w:w) ratio of reagent to DNA. After purification by spin column chromatography, both the labeled and unlabeled fragments were analyzed by 1% agarose gel electrophoresis. The rhodamine-labeled DNA fragments were visualized directly at 302 nm on a UV light box. Since this is not the optimal wavelength for detection of rhodamine, an exposure of 5 seconds was used for the photograph in Figure 3. The unlabeled fragments were stained with ethidium bromide.

Post_{IT}



Common Protease Inhibitors

	Specificity	Suggested stock and its stability	Suggested final concentration	Molecular Weight
Antipain dihydrochloride	Papain and trypsin	10 mM (6.8 mg/ml) in water. One month at -20°C	100 µM	678
Antithrombin III	Coagulation cascade serine proteases	10 mg/ml in water. One week at 4°C, pH 7-9.	1 Unit/ml with heparin present	65,000
α_1 -Antitrypsin (bovine plasma)	Broad range for serine proteases as well as collagenase and cathepsin G	>1 mg/ml in 100 mM Tris, pH 8.5, for one week at 4°C	10 µg/ml	54,000
Aprotinin (Trasylol)	Broad range for serine proteases, but not thrombin or Factor X	10 mg/ml (1.5 mM) in Tris buffer or water, pH 7-8. Stable for 6 months at -20°C.	1 µg/ml (150 nM)	6512
Benzamidine	Trypsin-like serine proteases	15 mg/ml (100 mM)	0.15-1.5 mg/ml (1-10 mM)	154
Bestatin	Amino peptidases and other exopeptidases	5 mg/ml (16 mM) in MeOH for 6 months at -20°C	50 µg/ml (162 µM)	308
Chymostatin	Chymotrypsin (α , β , γ , δ)	6 mg/ml (10 mM) in glacial acetic acid for one month at -20°C	6-60 µg/ml (10-100 µM)	608
E64	Broad range for cysteine proteases	1 mM (0.36 mg/ml) in water to 10 mM in 1:1 mix of ethanol/water (neutral pH) for one month at -20°C.	10-20 µM	357
EDTA	Metalloproteases and calpains	500 mM in water. Stable at room temp. You must raise the pH of this solution to dissolve the EDTA.	1-5 mM	372
Leupeptin	Trypsin-like serine and cysteine proteases	10 mM (4.6 mg/ml) in water for six months at -20°C.	10-100 µM	460
Pepstatin A	Acidic (aspartic) proteases	1 mM (0.7 mg/ml) in MeOH for one month at -20°C.	1 µM	686
PMSF Phenylmethylsulfonyl fluoride	Serine proteases	100 mM (0.175 g/10 ml) in 100% isopropanol. Stable for >6 months at room temperature	200 µM-1 mM.	174
Phosphoramidon	Thermolysin, collagenase, and other metalloendoproteases	5 mM (2.9 mg/ml) in water for one month at -20°C.	10-100 µM	580
TLCK L-1-chloro-3-(4-tosylamido)-7-amino-2-heptanone hydrochloride	Trypsin inhibitor, but also a limited number of other trypsin-like serine and cysteine proteases (but not chymotrypsin)	2 mM (0.74 mg/ml) in 1 mM HCl made fresh. Stable at 25°C	100 µM	369
TPCK L-1-chloro-3-(4-tosylamido)-4-phenyl-2-butanone	Chymotrypsin inhibitor, but also a limited number of other chymotrypsin-like serine and cysteine proteases (but not trypsin)	5 mM (1.76 mg/ml) in EtOH	200 µM	352
Trypsin inhibitor (soybean)	Inhibits trypsin, Factor Xa, plasmin and plasma kallikrein	1 mg/ml in water for six months at -20°C. Labile to heat and high pH.	1-10 µg/ml	20,100

Most recommended concentrations can be altered slightly in order to make solution preparation easier. For instance, Pepstatin A can be made up at 1 mg/ml instead and diluted appropriately.

A suggested basic protease inhibitor cocktail

Inhibitor	Specificity	Final Concentration
PMSF	Serine and Cysteine proteases	1 mM
E64	Cysteine proteases	10-20 µM
Leupeptin	Serine and Cysteine proteases	20 µM
EDTA	Metalloproteases	1-5 mM
Pepstatin	Acidic proteases	1 µM
Benzamidine	Serine proteases	2 mM
Phosphoramidon	Metalloproteases	10 µM

ER β : A New Member of the Steroid Receptor Superfamily

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The finding of another subtype of estrogen receptor, designated estrogen receptor β (ER β), provides a new focus for further understanding the mechanisms of estrogen regulation. The ER β gene, first cloned from a rat prostate cDNA library, encodes a protein of 485 amino acids with a predicted molecular mass of 54 kDa (1). Human ER β has also been cloned from testis (2) and mouse ER β from ovary (3). Compared to the 67-kDa ER α , human ER β (Figure 1) contains about 80 fewer residues in the N-terminal domain, but shares sequence homology of 96% and 58% in the DNA binding and ligand binding domains (LBD), respectively (2). The lack of sequence homology in the ER α and ER β transcriptional activation function domain 1 (AF-1) may play a role in differential responses to coactivators or ligands (2). The gene for ER β has been mapped to chromosome 12 in the mouse (3) and chromosome 14 in the human (4). The identification of another ER from a tissue not considered a target for estrogen action has fostered a number of recent studies examining the ligand interaction, transcriptional activation, tissue expression, and protein-protein interactions of ER β .

Sequence conservation in the LBD suggested that ER α and ER β share similar ligand binding specificity. Studies using *in vitro* transcribed full-length ER (1, 5) or an ER LBD fragment expressed from *E. coli* (6) demonstrated that both ER α and ER β bind 17 β -estradiol (E₂) with similar high affinity, although a report of slightly lower affinity E₂-ER β interaction has been noted (3). Competition assays using physiological estrogens including estrone, 17 α -estradiol, and estril or antagonists such as ICI-164384 and 4OH-tamoxifen indicated that these compounds bound to ER α and ER β with similar relative binding affinities, except that 17 α -estradiol bound with five times greater affinity to ER α than ER β (5). Estrogenic compounds such as diethylstilbestrol (DES), hexestrol, and dienestrol bound to both ER α and ER β with slight variations in their relative binding affinities

(5). Such differential ligand interactions may affect the transcriptional activation of genes controlled by estrogen responsive promoters.

ER α and ER β also display variations in their tissue expression. In the rat, ER β was found predominantly in prostate, ovary, lung, bladder, brain, uterus, and testis whereas ER α was detected in uterus, testis, pituitary, ovary, kidney, epididymis, and adrenal (5). ER β was detected in granulosa cells of primary, secondary, and mature follicles in the ovary by *in situ* hybridization (1, 7) and immunohistochemical studies (8). Treatment with gonadotropins down-regulated rat ovarian ER β mRNA (7). Comparison of ER α and ER β expression in the rat ovary indicated that ER β is the predominant subtype, which suggests an important role for ER β in follicular growth and oocyte development (7). ER β has also been detected in the hypothalamus of the rat (9, 10). The finding of ER β in neurons of the hippocampus, which does not contain high levels of ER α , suggests that ER β may play a role in E₂ action in these tissues (10).

In differentiating human osteoblasts, ER β mRNA expression increased approximately 10-fold during 21 days in culture

in the gastrointestinal tract (4). ER β expression in the human prostate was lower than that detected in the rat prostate (4). ER β is expressed in mouse mammary glands (12) and found in varying levels in human breast tumor biopsy samples and cultured human breast epithelial cells (13). In some cases, ER β was expressed in the same biopsy samples as ER α , which may add further complexity to the roles of ER in estrogen-induced regulation and hormone-independent breast cancers (4, 13).

ER β has been shown to bind specifically to the same consensus estrogen response element (ERE) sequences as ER α in the presence or absence of E₂ (3). Transient transfection studies indicated that ER β activated transcription of ERE-containing reporter genes in the presence of E₂ and that such induction was blocked by antagonists such as 4OH-tamoxifen, raloxifene, ICI-182780, or EM-652, the active metabolite of the novel antiestrogen EM-800 (3, 12). In contrast to ER α , 4OH-tamoxifen did not display partial agonistic activity with ER β (3). ER α agonistic activity has been mapped to AF-1 (14), and the lack of homology between ER α and ER β in the AF-1 domain may explain this difference (3). Interaction between the mouse ER β

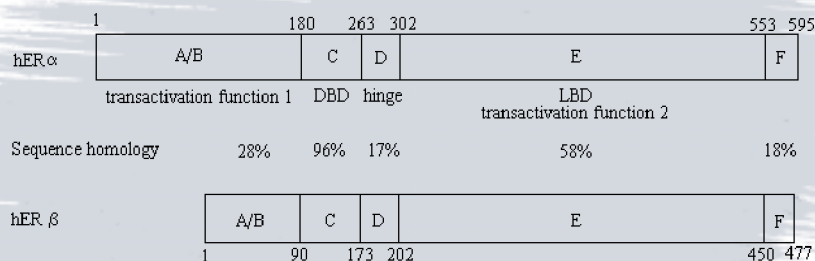


Figure 1

The structural and functional domains of human ER α and ER β . Domains A/B and E contain transcriptional activation function sequences, designated AF-1 and AF-2. The highly conserved DNA binding domain (DBD) is found in region C. Domain D contains the hinge region, and region E contains the ligand binding domain (LBD). The numbers adjacent to the schematics indicate the amino acid residue of the domain boundaries of each protein. The percentages of sequence homology between each domain of ER α and ER β are also shown (2, 23).

whereas ER α expression increased only 2-fold until day 10 and did not increase further (11). More quantitative studies are needed to determine mRNA and protein amounts in osteoblasts, but these findings may have significant implications for bone metabolism and osteoporosis therapeutics. Strong expression of human ER β was detected in testis and ovary by Northern blotting (4). Human ER β transcripts were also localized to the mucosa of the stomach, duodenum, colon, and rectum, unlike the rat where little ER β expression was found

LBD and the coactivator SRC-1 was enhanced in the presence of E_2 but not 4OH-tamoxifen, ICI-182780, or EM-652 (3, 12). However, ligand-independent activation of ER β , but not ER α , by SRC-1 was detected in transfected COS-1 and HeLa cells (3). Ras-mediated enhancement of transcription in the presence of E_2 has been observed for ER β (3) and ER α (15). When ER β serine 60 was mutated to abolish potential phosphorylation at this residue, the stimulatory effect of Ras was lost, suggesting that ER β is also regulated by the mitogen-activated protein kinase pathway (3). Studies of ligand-activated transcription of ER-regulated genes via AP-1 enhancer sites, which also bind the transcription factors Fos and Jun, have demonstrated a different role for ER β (16). In transient transfection experiments, ER β activated transcription of reporter genes containing AP-1 in the presence of ICI-164384, raloxifene, and tamoxifen, but repressed transcription in the presence of E_2 and DES (16). These results contrasted with those found using the single-point ER α mutant HEO at AP-1 sites (16). Further comparisons using the wild-type ER α are needed to clarify this new role for ER β at AP-1 sites.

The transgenic ER α -knockout mouse (ERKO) which is homozygous for a disrupted ER α gene (17) has provided a useful tool to examine ER β tissue distribution and function in comparison to the wild-type (WT) mouse. The ERKO mouse has been shown to survive to adulthood but is characterized by abnormal reproductive function and lower bone density. Interestingly, several estrogen responses remain in the ERKO mouse. Estrogen has been shown to play a role in suppression of vascular lesions from atherosclerosis or injury (18). When WT and ERKO mice were subjected to vascular injury, both

groups experienced increases in vascular medial area and smooth muscle cell proliferation, which were inhibited by E_2 administration to the same extent in both groups (19, 20). ER β expression was detected in the aorta of ERKO mice by RT-PCR (19). Further experiments are required to delineate the potential role of ER β in E_2 -mediated cardioprotective effects. By quantitative RNase protection assays, murine ER β mRNA has been found in the ovary, uterus, oviduct, prostate, epididymis, hypothalamus, and lung (21). Levels of ER β transcripts expressed in ovary, prostate, and epididymis were reduced in the ERKO mouse compared to WT, suggesting that expression or function of ER β may be dependent on ER α in some tissues (21).

Given the similarity in the ER LBD that also contains sequences necessary for ER α dimerization, several studies have examined whether ER β could homodimerize or heterodimerize with ER α . ER α and ER β have been shown to form homodimers and heterodimers that bind to ERE sequences in gel shift assays (22, 23, 24). ER α homodimers and ER α /ER β heterodimers formed complexes with DNA with a K_d of 2 nM, whereas ER β homodimers bound to DNA with 4-fold lower affinity (22). ER α mutants with decreased ability to homodimerize also formed heterodimers with ER β at a reduced level, suggesting that sequences important for ER α homodimerization also play a role in ER α /ER β heterodimerization (22). When ER α and ER β were transiently transfected in various ratios into cells in the presence of E_2 , ER α homodimers activated transcription to a

higher level than ER β homodimers and ER α /ER β heterodimers stimulated transcription to an intermediate level (22). Transcriptional activation by the three dimeric forms was reduced in the presence of 4OH-tamoxifen and ICI-182780 (22). Further studies to quantitate the amount of ER α /ER β heterodimers versus homodimers actually responsible for transcriptional activation would be quite useful in these studies where mixed populations are present. All three dimeric forms when bound to DNA also interacted with the coactivator SRC-1 in the presence of E_2 , although SRC-1 interaction with ER α /ER β heterodimers was less dependent on ligand occupancy (22). Mouse ER α and ER β were also shown to interact as heterodimers *in vivo* via a two-hybrid system (24). It will be interesting to see if an ER β splice variant that contains 54 additional nucleotides in the LBD and is found in the rat ovary and prostate (25) encodes an isoform with the ability to heterodimerize with ER α and function independent of ligand or as a dominant negative mutant.

The discovery of another ER has provided much excitement in the field of estrogen regulation and steroid receptor biology. The development of an ER β knockout mouse will be quite useful for further analyzing the function of ER β . In light of the recent findings describing ER β 's differential response to ligands, tissue distribution, and ability to heterodimerize with ER α , other exciting developments can be expected in the near future.

Circle response number 102



Mary S. Ozers



Jack Gorski

Photo by Jeff Miller, UW-Madison

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New PRODUCTS

Immunoassay Kits

- Procollagen
- Cadherins
- Fibronectin, fibronectin receptor
- Vitronectin, vitronectin receptor
- Osteocalcin, osteonectin
- Procollagen

TaKaRa's enzyme immunoassay (EIA) kits provide complete systems for the non-radioactive detection and quantitation of specific molecules. The kits can be used on most types of biological samples, such as plasma, serum, urine, and cell extracts. The kits are based on sandwich-type EIAs, using two monoclonal antibodies which recognize different epitopes on a target molecule. The first antibody is immobilized in microwell plates, while the second is supplied as a peroxidase conjugate. Samples are quantitated by addition of a colorimetric substrate and measurement in a standard EIA plate reader.

MESACUP™ Kinase Assay Kits

The MESACUP Kinase Assay Kit, developed by MBL, is an enzyme-linked immunosorbent assay (ELISA) that is as sensitive as radioactive methods, and is less affected by the presence of ATP in samples. This convenient, non-radioactive assay can be performed on crude cell extracts, column fractions, and purified enzyme. PanVera provides two formats: the MESACUP Protein Kinase Assay Kit (for PKC or PKA, Product # MBL 5230), and the MESACUP cdc2 Kinase Assay Kit (Product # MBL 5235). Both kits use antibodies to phosphorylated peptides to detect kinase activity. The kits include peroxidase-conjugated streptavidin and a colorimetric substrate for signal detection.

RetroNectin™ and RetroNectin Dish

RetroNectin (recombinant human fibronectin fragment CH-296) provides an easy, quick, and efficient method to enhance retroviral-mediated gene transfer into mammalian cells. The method is a convenient alternative to other retroviral gene transfer protocols, such as co-cultivation of target cells with virus-producing cells. The use of RetroNectin also avoids the need for polycations, which can be toxic to some cells.

RetroNectin (Product # TAK T100A, TAK T100B) is available in two sizes as a lyophilized powder for maximum versatility in designing experiments. The RetroNectin Dish (Product # TAK T110A) provides a time-saving alternative. Each 35mm dish (10 cm² area) is precoated with RetroNectin, eliminating the need to prepare solutions and coat plates before each use.

MEBSTAIN Apoptosis Detection Kit

Programmed cell death plays a major role in developmental biology and in the maintenance of homeostasis in higher organisms. The morphological changes that accompany programmed cell death are collectively referred to as apoptosis.

TaKaRa's MEBSTAIN Apoptosis Kit Direct (Product # MBL 8445) provides a sensitive and rapid method for detecting apoptosis. The kit is suitable for use in histochemistry, cytospin preparations, and flow cytometry. The protocol is based on the TdT-mediated dUTP nick end-labeling (TUNEL) method, which labels the 3'-OH ends of DNA fragments generated by apoptosis. The MEBSTAIN Apoptosis Kit Direct uses fluorescein-dUTP as the label, allowing detection of DNA fragmentation by fluorescence microscopy. The direct detection method eliminates the need for a secondary detection step with antibody conjugates, which is required with biotin-based systems.

New Product Ordering Information:

PCR and PCR-related:

TAK DS002	Fluorescence Differential Display Kit (Fluorescein version)	\$920
TAK DS003	Fluorescence Differential Display Kit (Rhodamine version)	\$920
TAK R025A	Enzyme Set - FDD	\$570
TAK 6622	Cloning-Sequencing Primer Set for FDD	\$320

Apoptosis:

MBL D0416	Anti-Fas Ligand Antibody, biotinylated, 50 tests (Flow Cytometry)	\$250
MBL 8445	MEBSTAIN Apoptosis Kit-Direct, 40 (histochemistry) or 66 (flow cytometry) assays	\$280
MBL 8441	MEBSTAIN Apoptosis Kit II, 40 (histochemistry) or 66 (flow cytometry) assays	\$280

gene transfer:

TAK T100A	RetroNectin™	0.5 mg	\$150
TAK T100B	RetroNectin™	2.5 mg	\$600
TAK T110A	RetroNectin™ Dish	10 plates	\$300

immunoassay kits:

MBL 5230	MESACUP™ Protein Kinase Assay Kit	96 assays	\$295
MBL 5235	MESACUP™ cdc2 Kinase Assay Kit	96 assays	\$295
TAK MK410	Universal Tyrosine Kinase Assay Kit	96 assays	\$250
TAK MK101	Procollagen Type I C-Peptide EIA (Precoated)	96 assays	\$480
TAK MK117	Human E-cadherin EIA Kit	96 assays	\$640
TAK MK015	Fibronectin EIA Kit	96 assays	\$480
TAK MK008	Fibronectin Receptor EIA Kit	96 assays	\$480
TAK MK002	Vitronectin EIA Kit	96 assays	\$480
TAK MK004	Vitronectin Receptor EIA Kit	96 assays	\$480
TAK MK111	Gla-Type Osteocalcin EIA Kit (Precoated)	96 assays	\$640
TAK MK013	Osteonectin/SPARC EIA Kit	96 assays	\$480
TAK MK006	Muscle-Type Calpastatin EIA Kit	96 assays	\$480
TAK MK007	Laminin EIA Kit	96 assays	\$480

* Additional kits available. Please contact PanVera.

New PRODUCTS

New Phase II Drug Metabolizing Enzymes

PanVera announces the availability of **Recombinant Human Sulfotransferase (SULT)** and **UDP Glycosyltransferase (UGT)** enzymes. These enzyme families play an important role in the biotransformation of xenobiotics. Their availability offers drug metabolism groups the tools required for the development of new *in vitro* methods for the evaluation of the pharmacokinetic properties of drugs.

Sulfotransferases (SULT)

We offer four Sulfotransferase enzymes as highly active cytosolic extracts: **SULT2A1 (STD)**, **SULT1E (STE)**, **SULT1A3 (STM)**, and **SULT1A1*2 (PST1)**. The Sulfotransferases (STs) are a class of enzymes that catalyze the sulfonation of many hormones, neurotransmitters, drugs, and other xenobiotic compounds using phosphoadenosine phosphosulfate (PAPS) as a sulfate donor.

UDP Glycosyl transferases (UGT)

Two new UDP glycosyltransferases, **UGT1A7** and **1A10**, join the 1A1 and 1A6 isozymes in our growing line of Phase II drug metabolizing enzymes. UGT **BACULOSOMES™** (microsomes) are produced from insect cells that are infected with a baculovirus containing a cDNA for human UGTs. There are approximately twelve known forms of human UGTs and their activity is defined by the conjugation of glucuronic to a wide variety of xenobiotic and endogenous substrates.

Screen-for-Competitors Kit ER-β

PanVera has incorporated its new human recombinant Estrogen Receptor-β (hrER-β) into an exciting Screen-for-Competitors Kit. Using the power of fluorescence polarization, this kit quickly assesses the ability of environmental compounds to bind ER-β. The procedure has a variety of applications, including endocrine disruptor screening, drug discovery, and steroid biochemistry research. The kit includes PanVera's intrinsically fluorescent estrogen (Fluormone™ ES1) which binds to hrER-β. A sensitive, non-radioactive, FP competition assay is used to determine the ability of the test compound to displace Fluormone ES1 from the ES1-hrER-β complex. A similar series of Screen-for-Competitors Kits is also available for ER-α.

New Product Ordering Information:

FP Screen-for-Competitors Kit Description*:

P2473	Estrogen Receptor-β	2 plates (192 assays)	\$ 360
P2474	Estrogen Receptor-β	10 plates (960 assays)	\$1,450
P2475	Estrogen Receptor-β	50 plates (4,800 assays)	\$5,670
P2313	Estrogen Receptor-α	2 plates (192 assays)	\$360
P2326	Estrogen Receptor-α	10 plates (960 assays)	\$1,450
P2338	Estrogen Receptor-α	50 plates (4,800 assays)	\$5,670

Drug Metabolizing Enzymes:

P2435	Sulfotransferase 2A1	250µg	\$195
P2436	Sulfotransferase E1	250 µg	\$195
P2437	Sulfotransferase 1A3	250 µg	\$195
P2438	Sulfotransferase 1A1*2	250 µg	\$195
P2447	UGT1A7 BACULOSOMES, rHuman	5 mg	\$125
P2472	UGT1A10 BACULOSOMES, rHuman	5 mg	\$125

* Each kit contains Fluormone ES1, Estrogen Screening Buffer, purified recombinant human ER, DTT, and instructions.

Q&A

I'm looking for a simple, quantitative method for studying DNA-protein binding.

While gel-shift assays, footprinting, and transcriptional activation provide information about the location or effect of protein binding to DNA, each of these methods has distinct limitations. Using fluorescence polarization (FP), DNA-protein interactions are observed in solution, in real-time, and in a homogeneous format. There is no need for filter binding, electrophoresis, precipitation, or any other sort of separation. This technique measures the change in molecular volume of a fluorescently-labeled DNA molecule as it binds to protein. The method requires very little sample (100 μ l), provides picomolar sensitivity, and is noninvasive. The samples can be remeasured under different solution conditions such as salt, temperature, or viscosity.

Is there an easy way to detect trace amounts of DNA in a high level of protein?

Yes. PanVera's Beacon[®] Total DNA Quantitation Kit can quantify 1 ng of DNA in protein solutions as concentrated as 4 mg/ml. The versatile, fluorescence-based kit includes the components to generate a standard curve via serial dilution of a DNA standard. A best-fit line is then plotted and used to determine the DNA concentration of unknown samples. Both high (3-200 ng) and low (0.098-25 ng) curves can be generated, depending on the DNA concentration of the sample.

Is there a way to make the reconstitution of purified cytochrome P450 enzymes easier?

Performance of classical reconstitution assays requires multiple pipetting and the preparation of fresh stock solutions making the whole process rather time consuming and involved. We have devised a simplified system, the RECO[™] System, which makes this process much easier and more reliable. The RECO System allows you to perform reconstitution assays with purified enzymes by simply combining the enzyme and buffer mixes. These mixes contain the necessary components for functional P450 activity. Simply add a substrate and start the reaction with NADPH (or a NADPH regenerating system). This "Mix & Metabolize" configuration is as easy to use as microsomes and offers the inherent advantages associated with purified proteins. The RECO System enzyme and buffer mixes are stable for 6 months at -80° C and for at least two weeks at -20° C and 4° C. The mixes are also stable at room temperature making them well suited for high-throughput formats. RECO Kits are available for CYP1A2, 2C9, 2E1, and 3A4.

Customer Service

Monday-Friday
7:00 a.m.-6:00 p.m. CST

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608-233-5050

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Published quarterly by
PanVera Corporation
545 Science Drive
Madison, WI 53711
USA
www.panvera.com



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