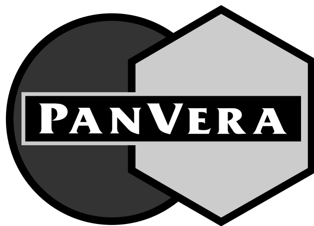

The Protein Company™

Postings

Issue 6 PanVera Corporation

March 2000

"The most exciting phrase to hear in science, the one that heralds new discoveries, is not 'Eureka!' (I've found it!), but 'That's funny'..."

Isaac Asimov

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The new PanVera Catalog 2000 is now available, containing information on new products and assays—proteins, fluorescence polarization, PCR, molecular and cell biology, and more. If you have not yet received your copy, please e-mail lit@panvera.com or call 800.791.1400.

HybQUEST™ Complete (DNP) System: Nonradioactive Labeling, Hybridization, and Detection

Mary-Anne V. Watt, Paul M. Slattum, Kira J. Machnik, and James E. Hagstrom
Mirus Corporation

Introduction

Hybridization analysis of DNA is important in the characterization of cloned genes, investigation of genetic diseases, detection of pathogens, and many other areas of science. Here we demonstrate the HybQUEST™ Complete (DNP) System, which allows covalent modification of probe DNA using *Label* IT® DNP Reagent and optimized chemiluminescent detection of hybridized DNP-labeled probes. The HybQUEST™ System provides an ultrasensitive, easy-to-use, and complete nonradioactive hybridization and detection kit for use in membrane-based applications.

Nonradioactive hybridization and detection is an attractive alternative to radioactive methods because it offers safer handling and storage. However, radioactive detection has traditionally offered higher sensitivity and lower background than many nonradioactive methods. As a result of recent improvements in chemiluminescent detection techniques, sensitivity of nonradioactive probe detection now compares favorably with that of radioactive methods.

Mirus' new HybQUEST™ Complete (DNP) System was designed to take advantage of these recent advances by providing exceptional detection sensitivities with easy-to-use components. At the core of the HybQUEST™ System is the *Label* IT® Reagent—a novel chemical DNA labeling reagent designed to overcome many of the limitations encountered in both enzymatic and other nonenzymatic methods for generating nonradioactive hybridization probes. *Label* IT® Reagent efficiently promotes the one-step covalent attachment of dinitrophenyl (DNP) labels to any nucleic acid species. The simple one-hour labeling reaction allows the user to easily scale the reaction and to optimize the labeling density for a particular hybridization application. The HybQUEST™ System permits detection of the DNP-labeled DNA probes using a highly-specific antiDNP antibody that provides exceptional signal-to-noise ratios. Each kit includes DNA controls and a DNP-labeled DNA molecular size marker, which can be visualized by both ethidium bromide staining of the gel and chemiluminescent detection of the blot.

To examine the versatility and sensitivity of the HybQUEST™ System, we prepared DNA probes and hybridized them to target sequences in a variety of commonly used applications. We then detected the hybridized probes using the alkaline phosphatase-conjugated antiDNP antibodies and Lumi-Phos® Plus chemiluminescent substrate provided with the kit. We assessed the results for: (1) the effectiveness of the kit in a range of hybridization applications, (2) the sensitivity of detection, and (3) the ability to strip and reprobe the blots.

Figure 1. One-step, nonradioactive DNP labeling of probe DNA. Agarose gel electrophoresis of approximately 0.2 µg of a ~900 bp glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe before (lane 2) and after (lane 3) labeling with the *Label* IT® DNP Reagent. The 1% gel was stained with ethidium bromide. Lane 1 contains 1 µg of a 1 kb DNA ladder.

Probe Labeling

We performed a standard labeling reaction by incubating probe DNA with *Label* IT® Reagent for one hour at 37°C. Following purification, we examined the labeled probe by agarose gel electrophoresis (Figure 1). The modified DNA demonstrates a marked reduction in electrophoretic mobility compared with unlabeled DNA. Alternatively, the probe may be examined by immunochemical detection of the DNP label using a dot blot procedure (not shown); however, agarose gel electrophoresis permits assessment of the integrity of the probe following the labeling reaction. Based on our results, we estimated that the density of DNP tags on the DNA, following a standard labeling reaction, is approximately 40-60 bp per label (data not shown).

Hybridization and Detection

We performed all hybridizations according to protocols provided with the kit using 20 ng/mL of denatured probe for 16 hours at 42°C in a formamide-based hybridization buffer (supplied). We detected the DNP epitope using the kit's alkaline phosphatase-conjugated antiDNP antibody and Lumi-Phos® Plus chemiluminescent substrate. We exposed the membrane to Kodak Biomax® ML film and manually processed it using Kodak GBX reagents.

The HybQUEST™ System functioned effectively in all hybridization formats we tested, including the often dirty (i.e., high-background) colony-lift hybridizations (Figure 2). We obtained detection sensitivities in the femtogram range (Figures 3 and 4) and were easily able to strip the hybridized probes and reprobe the target DNA (Figure 5).

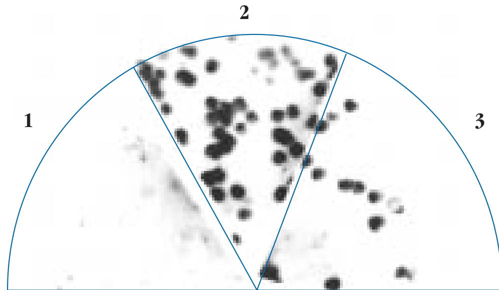

Figure 2. Colony lift hybridizations. Bacterial colony lifts were prepared, using positively charged nylon membrane, from three different *E. coli* cultures. **Section 1:** DH10b/pBS-GAPDH; **Section 2:** DH10b/pCI-luciferase; **Section 3:** a mixed population of DH10b/pBS-GAPDH and DH10b/pCI-luciferase. Segments of each colony lift were hybridized using a DNP-labeled luciferase probe.

100 ng 10 ng 1 ng 100 pg 10 pg 1 pg 0.1 pg

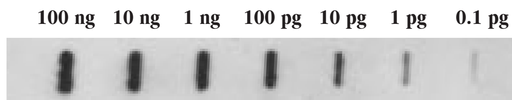

Figure 3. Slot blot hybridization. Target DNA was blotted to a positively charged nylon membrane in 10-fold serial dilutions from 100 ng to 0.1 pg. The slot blot was hybridized with a DNP-labeled complementary DNA probe.

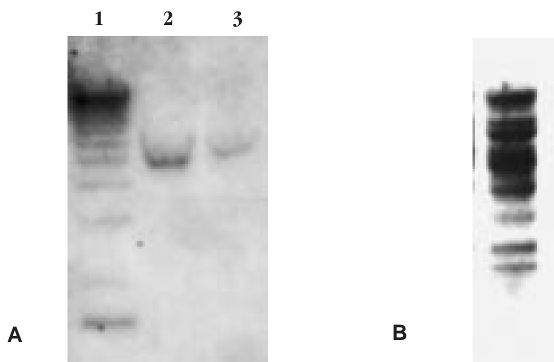

Figure 4. Genomic Southern hybridizations. Panel A: Detection of the single copy gene (human Factor IX)[1] in 10 µg (lane 2) and 5 µg (lane 3) of *Eco*R I-digested human genomic DNA using a 1.4 kb DNP-labeled cDNA probe. Lane 1 is a DNP-labeled 1 kb ladder. **Panel B:** Hybridization analysis of human genomic DNA (5 µg) using a DNP-labeled human GAPDH probe (900 bp as shown in Figure 1). GAPDH has been characterized as a single expressed locus with one pseudogene and 15 related loci.[2]

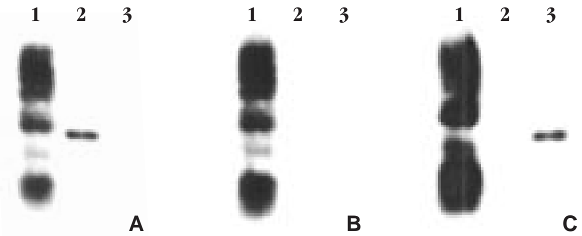

Figure 5. Stripping and reprobing Southern blots. The target DNA was resolved by agarose gel electrophoresis and transferred to a positively charged membrane: lane 1, DNP-labeled 1 kb ladder, lane 2, 10 pg of 1.7 kb luciferase PCR product, and lane 3, 10 pg of 1.4 kb Factor IX PCR product. **Panel A:** Hybridization results using the DNP-labeled luciferase DNA as probe. **Panel B:** Detection of the DNP epitopes on the same membrane following a standard SDS stripping procedure. **Panel C:** Hybridization of the same membrane with a DNP-labeled Factor IX probe.

Conclusion

Many common molecular biology applications, such as Southern, dot, and slot blotting and colony- or plaque-lift hybridization, require the detection of specific DNA hybridization events. Factors involved in the generation and detection of strong, specific, and reproducible hybridization signals include the nature of the probe, the type and density of the label, the characteristics of the hybridization solution, the specificity and avidity of antibodies, and the kinetics of the enzyme used in chemiluminescent detection. The HybQUEST™ Complete (DNP) System optimizes many of these key parameters and provides ultrasensitive, nonradioactive hybridization and detection in a complete, easy-to-use kit. This series of experiments illustrates the sensitivity and specificity of DNP-labeled DNA probes in a variety of hybridization applications using the HybQUEST™ System.

References

1. Jagadeeswaran, P., *et al.* (1984) *Somat. Cell Molec. Genet.* **10**: 465-473.
2. Benham, F. J. and Povey, S. (1989) *Genomics* **5**: 209-214.

Mary-Anne Watt

ORDERING INFORMATION

Product No.	Product Name	Quantity	Price
MIR 6000	HybQUEST™ Complete (DNP) System	1 kit	$450
Kit includes the following components that may also be ordered separately:			
MIR 6800	HybQUEST™ *Label* IT® (DNP) Kit	5 x 2 µg rxns	$175
MIR 6010	HybQUEST™ Hybridization and Detection (DNP) Kit	10 blots	$295

HybQUEST™ *Label* IT® Kits are also available for labeling probes with fluorescein (MIR 6200), digoxin (MIR 6300), and biotin (MIR 6400).

ZAP-70 and Epidermal Growth Factor Receptor: Protein Tyrosine Kinase Assays for Drug Discovery

Gregory Parker and Tong Law
PanVera Corporation

Introduction

ZAP-70 and epidermal growth factor (EGF) receptor belong to the family of protein tyrosine kinases (PTKs) and are key regulators of cell-signaling events. Mutations in genes encoding these proteins are responsible for serious disease states in humans. In this report, we examine the activity and inhibition of PanVera's new ZAP-70 and EGFR using our PTK Assay. This sensitive, fluorescence polarization (FP)-based method can be used for high-throughput screening of activity modulators and potential drug therapies.

We first examined ZAP-70, a protein tyrosine kinase essential for T-cell antigen receptor (TCR)-mediated signal transduction. Upon encountering a peptide antigen presented in conjuction with major histocompatibility complex (MHC) class II proteins, the TCR initiates a biochemical cascade involving the protein tyrosine kinases Fyn, Lck and ZAP-70. This cascade results in T-cell differentiation and activation.[1] Mutations of the ZAP-70 gene are responsible for a rare, autosomal recessive form of severe combined immunodeficiency syndrome (SCID) in humans. Additionally, the absence of ZAP-70 eliminates the response to antigen or mitogen stimulation and disables T-cell proliferation and activation.[2]

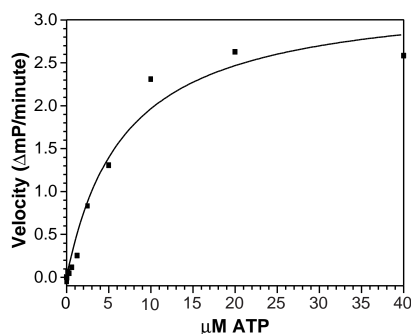

Figure 1. Binding of ATP to ZAP-70. Samples of ATP were serially diluted in a round-bottom, black 96-well plate. Final reaction conditions were 0.21 μg/mL ZAP-70, 20 mM HEPES (pH 7.4), 5 mM $MgCl_2$, 2 mM $MnCl_2$, 50 μM Na_3V0_4, and 50 μg/mL poly[Glu,Tyr] 4:1. After an 80-minute incubation at room temperature, EDTA was added to a final concentration of 20 mM to stop the reaction. Kinase activity was measured by adding an equal volume (50 μL) of PTK Detection Mix (PanVera) to each well. After a 5-minute incubation, the fluorescence polarization of each well was read on a TECAN Polarion instrument. Data were plotted and analyzed using GraphPad Prism® software.

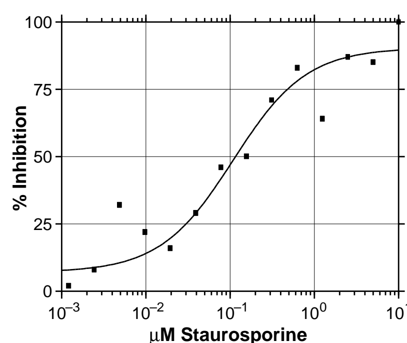

Figure 2. Inhibition of ZAP-70 activity using staurosporine. Samples of staurosporine were serially diluted in a round-bottom 96-well plate. Final reaction conditions were 0.21 μg/mL ZAP-70, 20 mM HEPES (pH 7.4), 5 mM $MgCl_2$, 2 mM $MnCl_2$, 50 μM Na_3VO_4, 50 μg/mL poly[Glu,Tyr] 4:1, 2% DMSO, and 5 μM ATP. After a 2-hour incubation at room temperature, EDTA was added to a final concentration of 20 mM to stop the reaction. Reaction products were assayed by adding an equal volume (50 μL) of PTK Detection Mix (PanVera) to each well. After a 5-minute incubation, the fluorescence polarization of each well was read on a TECAN Polarion instrument. Percent inhibition was calculated, and nonlinear regression analysis was performed using GraphPad Prism® software.

Next, we studied epidermal growth factor (EGF) receptor, an autophosphorylating cell-surface receptor possessing intrinsic tyrosine kinase activity. Numerous studies have shown that the EGF receptor is important in cell-growth control. The intrinsic tyrosine kinase activity is required for many of its biological effects.[3] In humans, EGF receptor overexpression and/or mutation has been associated with squamous cell carcinomas, glial tumors, and poor mammary tumor differentiation and grade.[4-8]

We assessed kinase activity for these two receptors using PanVera's PTK Assay. This homogeneous assay uses fluorescence polarization to quantitate the reaction product of a tyrosine kinase reaction. The system is based upon competition—phosphopeptides and fluorescein-labeled (F-) phosphopeptides generated during a kinase reaction competitively bind to antiphosphotyrosine antibodies. Without a kinase reaction, a significant portion of the F-phosphopeptide tracer will be bound by antibody, resulting in high polarization. Following a kinase reaction, the antibodies bind to the reaction products and the F-phosphopeptide tracer. The increase in free tracer decreases the fluorescence

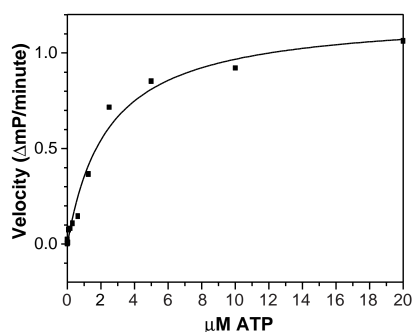

Figure 3. Binding of ATP to EGFR. Samples of ATP were serially diluted in a round-bottom, black 96-well plate. Final reaction conditions were 0.3 U/μL EGFR, 20 mM HEPES (pH 7.4), 5 mM $MgCl_2$, 2 mM $MnCl_2$, and 10 μg/mL poly[Glu,Tyr] 4:1. After a 2-hour incubation at room temperature, EDTA was added to a final concentration of 20 mM to stop the reaction. Kinase activity was measured by adding an equal volume (50 μL) of PTK Detection Mix (PanVera) to each well. After a 5- minute incubation, the fluorescence polarization of each well was read on a TECAN Polarion instrument. Data were plotted and analyzed using GraphPad Prism® software.

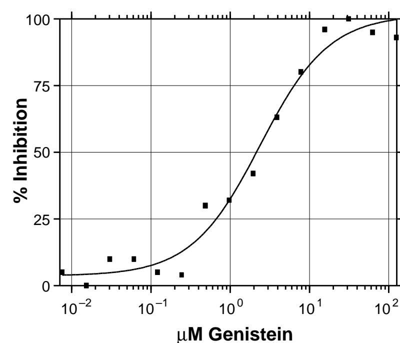

Figure 4. Inhibition of epidermal growth factor receptor activity using genistein. Samples of genistein were serially diluted in a round-bottom 96-well plate. Final reaction conditions were 0.015 U/μL EGFR, 20 mM HEPES (pH 7.4), 5 mM $MgCl_2$, 2 mM $MnCl_2$, 10 μg/mL poly [Glu,Tyr] 4:1, 5 μM ATP, and 0.5% DMSO. After a 4-hour incubation at room temperature, EDTA was added to a final concentration of 40 mM to stop the reaction. Kinase activity was measured by adding an equal volume (50 μL) of PTK Detection Mix (PanVera) to each well. After a 5-minute incubation, the fluorescence polarization of each well was read on a TECAN Polarion instrument. Percent inhibition was calculated, and nonlinear regression analysis was performed using GraphPad Prism® software.

polarization of the sample. The resulting change in FP is directly related to the amount of PTK activity.

Results

As shown in Figure 1, we used the PTK Assay to examine the binding kinetics of ATP and ZAP-70. After we obtained the change in polarization (mP) per minute, which is equal to the reaction velocity, we averaged the data for each ATP concentration. We performed nonlinear regression analysis using Graphpad Prism® software and determined the K_m to be 6.90 μM. Others have reported the apparent K_m to be ~3 μM.[9] We then investigated the inhibition of ZAP-70 activity using the broad-spectrum kinase inhibitor staurosporine (Figure 2). Upon analysis of these data, we determined the IC_{50} for staurosporine to be 112.7 nM.

We performed a similar set of experiments using EGF receptor. From the data shown in Figure 3, we determined the K_m for ATP binding by EGFR to be 2.42 μM, which is in good agreement with previously performed experiments demonstrating a K_m value of 4.68 μM.[10] We then examined the inhibition of EGF receptor activity using genistein (4,5,7-trihydroisoflavone) and determined the IC_{50} to be 2.39 μM, as illustrated in Figure 4. This value is in agreement with previous observations.[10]

Summary

Receptor binding and signal transduction are now the focus of many drug discovery efforts, including those involving high-throughput screening. As regulators of key cell-signaling events, ZAP-70 and EGF receptor are becoming increasingly important targets for cellular research and drug development. In these experiments, we have demonstrated that ZAP-70 and EGF receptor can be used with PanVera's Protein Tyrosine Kinase Assay to provide sensitive detection of kinase activity and inhibition. These products should prove useful in the examination of T-cell activation and cell-growth regulation, which may lead to the development of novel pharmacologic agents.

References

1. Chan, A.C., *et al.* (1994) *Ann. Rev. Immunol.* **12**: 555-592.
2. Arpaia, E., *et al.* (1994) *Cell* **76**: 947-958.
3. Carpenter, G. and Cohen, S. (1990) *J. Biol. Chem.* **265**: 7709-7712.
4. Wong, A., *et al.* (1992) *Proc. Natl. Acad. Sci. USA* **89**: 2965-2969.
5. Yamamoto, T., *et al.* (1986) *Cancer Res.* **46**: 414-416.
6. Libermann, T.A., *et al.* (1985) *Nature* **313**: 144-147.
7. Cowley, G., *et al.* (1984) *Cancer Cells* **1**: 5-10.
8. Klijn, J.G.M., *et al.* (1992) *Endo. Rev.* **13**: 3-17.
9. Isakov, N., *et al.* (1996) *J. Biol. Chem.* **271**: 15753-15761.
10. Hubler, L., *et al.* (1992) *Biochem. J.* **281**: 107-14.

Gregory Parker

For additional information on PanVera's HTS assays, see the April issue of the Journal of Biomolecular Screening, *or visit our website at: www.panvera.com*

ORDERING INFORMATION

Product No.	Product Name	Quantity	Price
P2628	EGF Receptor, Human	10 U	$300
P2782	ZAP-70, rHuman	20 μg	$135
P2650	Protein Tyrosine Assay Kit, Beacon®	100 assays	$150
P2651	Protein Tyrosine Assay Kit, CoreHTS™	1,000 assays	$1,195

New Products

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Cytochrome P450 2D6

Purified, Recombinant Human, Histidine-Tagged

Cytochrome P450 2D6 is the latest addition to PanVera's line of purified, recombinant human P450s. CYP2D6 is histidine-tagged and produced in *E. coli.* As with PanVera's other P450s, the N-terminus has been modified to allow expression in *E. coli*, but these changes do not cause any significant differences in substrate specificity. The advantages of using purified cytochrome P450 enzymes include the lack of interfering activities present in microsomal or tissue samples, as well as the flexibility to optimize component ratios of P450, NADPH-P450 reductase (Product No. P2309), and Cytochrome b_5 (Product No. P2252) for specific applications.

Rac-1

Recombinant Human

Rac-1 is a small GTP-binding protein that is a member of the *ras* gene superfamily and Rho family of proteins. Rac-1 protein is involved in the regulation of a variety of signaling pathways that control cell growth and differentiation. It has been shown to mediate cell membrane ruffling and actin stress fiber formation, regulate nuclear factors in activated T cells, and regulate the JNK and MAP kinase pathways. Rac-1 is also required for oncogenic ras-mediated changes of the actin cytoskeleton in fibroblasts and mutated forms of the protein can act as a weak oncogene, inducing malignant transformation of cells. PanVera's Rac-1 is a 20.5 kDa, untagged, recombinant human protein and is an authentic substrate for geranylgeranyltransferase (Product No. P2617).

ZAP-70

Histidine-Tagged, Recombinant Human

ZAP-70 is a cytosolic protein tyrosine kinase that is a member of the Syk family of proteins. ZAP-70 is expressed exclusively in T cells and natural killer cells and is required for T-cell receptor activation, which triggers an immune response. ZAP-70 is activated when the protein tyrosine kinase Lck phosphorylates the tyrosine residues on ZAP-70. Phosphorylation of ZAP-70 is crucial to the recruitment of additional molecules, such as Vav, Ras, and PLC γ-1, to the membrane-associated signaling complex and for the induction of downstream signaling events. Mutation of the ZAP-70 genes results in a form of severe combined immunodeficiency syndrome (SCID) in humans. PanVera's recombinant ZAP-70 is a 70 kDa, full-length, human protein expressed with a histidine tag in baculovirus-infected insect cells. (For information on the use of ZAP-70 with PanVera's Protein Tyrosine Kinase Assay, see the article on p. 4.)

Vascular Endothelial Growth Factor

Recombinant Human

$VEGF_{165}$ is a secreted homodimeric protein that stimulates endothelial cell growth, angiogenesis and capillary permeability. VEGF is distinct among other growth factors in that its mitogenic activity is specific for endothelial cells and it is the only growth factor that stimulates vascular permeability. The physiological importance of VEGF includes development of normal vascularized tissues, wound healing, and development of human cancers and other diseases. PanVera's $VEGF_{165}$ is the human 165 amino acid VEGF variant, expressed in baculovirus-infected insect cells. It is greater than 95% pure and is tested for endotoxin.

NEW PRODUCT ORDERING INFORMATION

Product No.	Product Name	Quantity	Price
P2774	Cytochrome P450 2D6	150 µg	$185
P2773	Rac-1, Recombinant Human	20 µg	$220
P2782	ZAP-70, Histidine-tagged, Recombinant Human	20 µg	$135
P2654	$VEGF_{165}$	10 µg	$250
MIR 6000	HybQUEST™ Complete (DNP) System	1 kit	$450
MIR 6800	HybQUEST™ *Label* IT® (DNP) Kit	5 x 2 µg rxns	$175
MIR 6010	HybQUEST™ Hybridization and Detection (DNP) Kit	10 blots	$295
MIR 2200	*Trans*IT®- Insecta Reagent	1 mL	$185
MIR 2204		0.4 mL	$120
MIR 2205		5 x 1 mL	$833
MIR 2206		10 x 1 mL	$1,573
TAK 1223A	*Bst*T107 (*Hgi*C I, *Ban* I)	1,000 U	$60
TAK 1223B	*Bst*T107 (*Hgi*C I, *Ban* I)	5,000 U (A x 5)	$240
TAK 1196A	*Vpa*K11 B I (*Bfa* I, *Ava* II)	300 U	$50
TAK 1196B	*Vpa*K11 B I (*Bfa* I, *Ava* II)	1,500 U (A x 5)	$200
TAK 1095A	*Xsp* I (*Bfa* I, *Mae* I)	500 U	$60
TAK 1095B	*Xsp* I (*Bfa* I, *Mae* I)	2,500 U	$240
TAK 2910A	Micrococcal Nuclease	15,000 U	$125
TAK 2311A	RNase Inhibitor (Porcine Liver)	5,000 U	$80
TAK 2311B	RNase Inhibitor (Porcine Liver)	25,000 U (A x 5)	$320
TAK 2311S	RNase Inhibitor (Porcine Liver)	2,500 U	$50
TAK 3415A	Wide-Range DNA Ladder	100 tests	$133
TAK 3604	pAUR 135 DNA	20 µg	$250
TAK 3605	pAUR 136 DNA	20 µg	$250
TAK 3650	pDON-A1 DNA	20 µg	$240
TAK 6624	Differential Display Kit		$667
TAK 6625	Differential Display Kit (Fluorescein version)		$667
TAK 6626	Differential Display Kit (Rhodamine Kit)		$667
TAK R024A	Enzyme Set-DD		$383
TAK R025A	Enzyme Set-FDD		$567
TAK RR010A	One Shot Insert Check PCR Kit		$125
TAK RR201	PCR Screening Kit for GM Soybean, Version 2.0	48 tests	$1,000
TAK RR202	PCR Screening Kit for GM Maize, Version 2.0	48 tests	$1,000
TAK 9092	DNA Extraction Kit for GMO Detection	100 tests	$200
Related Products			
P2309	NADPH-P450 Reductase	200 µg	$185
P2252	Cytochrome b_5	100 µg	$150
P2617	Geranylgeranyltransferase I*	25 µg	$250
MIR 6200	HybQUEST™ *Label* IT® (Fluorescein) Kit	5 x 2 µg rxns	$175
MIR 6300	HybQUEST™ *Label* IT® (Digoxin) Kit	5 x 2 µg rxns	$175
MIR 6400	HybQUEST™ *Label* IT® (Biotin) Kit	5 x 2 µg rxns	$175

* Recombinant GGTase is covered by U.S. Patent No. 5,789,558 issued to Merck & Co., Inc. Rights have been granted to PanVera Corporation for research use only.

New Products

HybQUEST™ Complete (DNP) System

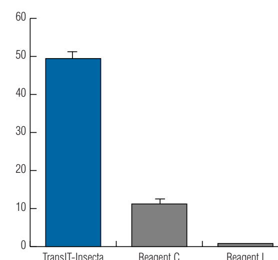
For sensitive, nonradioactive labeling, hybridization and detection of specific DNA hybridization events

The HybQUEST™ Complete System, developed by Mirus Corporation, provides all the reagents necessary for optimal labeling, hybridization, and chemiluminescent detection of dinitrophenyl (DNP)-labeled DNA probes. It is designed for sensitive nonradioactive detection of specific DNA hybridization events in membrane based applications, including Southern blots, bacterial colony and plaque hybridizations, and dot or slot blots. The key component of this kit is the proprietary *Label* IT® technology. *Label* IT® Reagent facilitates the one-step, nondestructive, nonradioactive covalent labeling of DNA within minutes. Since *Label* IT® Reagent directly modifies the starting DNA material, the labeling reaction is less variable than other methods. The labeling procedure is easily controlled to modify the density of labels on the probe and is highly sensitive.

*Trans*IT®-Insecta Transfection Reagent

For superior transfection efficiency in insect cell lines

*Trans*IT®-Insecta Transfection Reagent is the newest innovation from the gene transfer specialists at Mirus Corporation. The novel lipid mixture is specifically designed for superior transfection efficiencies in insect cell lines. The unique combination of lipids makes *Trans*IT®-Insecta Reagent ideal for expressing genes in insect cells and transfecting recombinant baculovirus expression systems into Sf9, Sf21, or High Five™ and S2 cells.



Frequently Asked Questions

Is there a way to increase my transfection efficiencies without complicating my protocol?

Yes. *Trans*IT® Reagents effectively deliver nucleic acids to cells grown with or without serum. In many cases you will see a decided increase in transfection efficiency when the cells are allowed to grow on serum-containing media. It is important to form your DNA/*Trans*IT® Reagent complexes in a serum-free environment and then transfer the complexes to the cells. Typically, there is no need to change the media, thus reducing the number of steps in the protocol. We recommend testing serum-free versus serum-containing media as a part of your optimization experiments.

I could not find my favorite *Trans*IT® Transfection Reagent in the PanVera 2000 Catalog's product number index. Is it still available?

Most likely yes. In order to provide more a more versatile product line, we have reconfigured the packaging of our *Trans*IT® Reagents. Here are the current part numbers, volumes and prices. If you are accustomed to using the 0.6 mL size of *Trans*IT® -LT1 or -LT2 Reagent, you will notice the cost per volume of our 1 mL size has decreased! If you are accustomed to using *Trans*IT® 100 Reagent, PanVera Technical Services can make specific recommendations for your cell line. Often, *Trans*IT® -Insecta Reagent may be appropriate as an alternative reagent for nonstandard cell lines. *Trans*IT® -LT1 and -LT2 Reagents may be used to transfect directly in serum (see previous question). If you have any questions or concerns, please contact us at: info@panvera.com

Product	Product No.	Quantity	Price
*Trans*IT® LT1	MIR 2300	1 mL	$170
	MIR 2304	0.4 mL	$110
	MIR 2305	5 x 1 mL	$765
	MIR 2306	10 x 1 mL	$1,445
*Trans*IT® LT2	MIR 2400	1 mL	$170
	MIR 2404	0.4 mL	$110
	MIR 2405	5 x 1 mL	$765
	MIR 2406	10 x 1 mL	$1,445
*Trans*IT® 100	This product has been discontinued.		
*Trans*IT®- Insecta	MIR 2200	1 mL	$185
	MIR 2204	0.4 mL	$120
	MIR 2205	5 x 1 mL	$833
	MIR 2206	10 x 1 mL	$1,573
*Trans*IT® PanPak	MIR 2510	200 µL each LT1, LT2, Insecta	$110

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Restriction Enzyme Sensitivity to *dam* and *dcm* Methylation

DNA prepared from most commmon strains of *E. coli* may prove resistant to cleavage by certain restriction enzymes. This can often be caused by site-specific methylation. Two types of methylases are commonly encountered: the *dam* methylase, which methylates the N6 position of the adenine residues in the sequence GATC, and the *dcm* methylase, which methylates the internal cytosine residues in the sequences CCWGG at the C5 position. The sensitivity of Takara's enzymes to these types of methylation is listed below.

Enzymes blocked by overlapping *dam* methylation

Enzyme	Site
Cla I	ATCGAT(C)
Fba I	TGATCA
Mbo I	GATC
Mbo II	GAAGA(TC)
Mfl I	RGATCY
Nru I	TCGCGA(TC)
*Tth*HB8 I	TCGA(TC)
Xba I	TCTAGA(TC)

Enzymes not blocked by overlapping *dam* methylation

Enzyme	Site
*Bam*H I	GGATCC
Bgl II	AGATCT
Pvu I	CGATCG
*Sau*3A I	GATC

Enzymes blocked by overlapping *dcm* methylation

Enzyme	Site
Apa I	GGGCCC(WGG)
Ava II	GGWCC(WGG)
Bal I	TGGCCA(GG)
*Cfr*13 I	GGNCC(WGG)
Eae I	YGGCCA(GG)
*Eco*O109 I	RGGNCCT(GG)
Sfi I	$GGCCN_5GGCC$(WGG) or $GGCCWGGN_2GGCC$
Stu I	AGGCCT(GG)
*Van*91 I	$CCAGGN_3TGG$

Enzymes not blocked by overlapping *dcm* methylation

Enzyme	Site
*Bam*H I	GGATCC
Bbe I	GGCGCC
*Bst*P I	GGTNACC
*Bst*X I	CCAN4CCTGG
*Eco*O65 I	GGTNACC
Hae III	GGCC
*Hin*1 I	GRCGYC
Kpn I	GGTACC
Mva I	CCWGG

M = A or C; K = G or T; N = A, C, G, or T;
R = A or G; Y = C or T; W = A or T; S = G or C

Come and see us...

PanVera will be attending the following meetings this year, exhibiting information on our newest and most exciting products and services. Please stop by!

American Association for Cancer Research (AACR)	April 1-5	San Francisco, CA	Booth # 847
Biotechnology Vendor Showcase 2000	April 13	Los Angeles, CA	Booth # 27
American Society for Microbiology (ASM)	May 21-25	Los Angeles, CA	Booth # 1049
Endocrine Society (ENDO 2000)	June 21-24	Toronto, ON	Booth # 1037
Society for Biomolecular Screening (SBS)	September 6-9	Vancouver, BC	
Land O' Lakes Drug Metabolism Conference	September 11-15	Devil's Head, WI	
NIH Research Festival ("Tent Show")	October 12-13	Bethesda, MD	
International Society for the Study of Xenobiotics (NA-ISSX)	October 24-28	Indianapolis, IN	Booth # 18