

Chromatin Immunoprecipitation using General Transcription Factors

Active Motif's new ChIP-IT Kit for chromatin immunoprecipitation (ChIP) enables researchers to get easy access to one of proteomics' powerful new tools for analyzing genome regulation. ChIP involves the identification of DNA fragments that are bound by a particular transcription factor. The fragments of interest are analyzed by semi-quantitative PCR using fragment-specific PCR primers or by hybridization to gene arrays. The power of ChIP lies in the combination of specificity (immunoprecipitation), sensitivity (PCR) and screening power (array profiling). No other technique can offer you such a versatile combination.

However, ChIP analyses can be technically challenging and difficult to validate unless performed using well-proven reagents. To make validating your ChIP reagents easier, the new ChIP-IT Kit contains antibodies and controls that have been proven to work effectively in ChIP. Plus, ChIP-IT antibodies are directed against general transcription factors so you get a more accurate analysis of promoter activity than is possible with histone-based ChIP kits. The new ChIP-IT Kit is the only kit available today that contains proven antibodies, reagents and controls, so you can get the best results possible, whatever your needs.

continued on page 5

Colorimetric Quantitation of MAPK-regulated Transcription Factors

Active Motif's new TransAM™ MAPK Family Kit makes it possible to rapidly profile the levels of six different transcription factors that are regulated by MAPK kinase activity. In one simple experiment, you can assay the levels of activation obtained under different growth and stimulation conditions. Because TransAM is an ELISA-based colorimetric method*, it eliminates the use of radioactivity and provides quantitative results in less than five hours. This makes TransAM faster, more sensitive and simpler than other techniques for studying transcription factors, like gelshift and Western blotting. And, the TransAM method makes it possible to study stimulated tissue samples.

Downstream MAPK detection

Activation of Mitogen Activated Protein Kinase (MAPK) involves a three kinase cascade consisting of a MAP kinase kinase (MAPKKK or MEKK) that activates a MAP/ERK kinase (MAPKK or MEK). This stimulates a phosphorylation-dependent increase in the activity of the MAP kinase. Upon activation, MAP kinases phosphorylate a variety of intracellular targets including transcription factors. The TransAM MAPK Family Kit directly assays the transcription factors ATF-2, c-Jun, c-Myc, Elk-1, MEF2 and STAT (Figure 1), all of which are regulated by MAP kinase cascades and are key markers in cell signaling.

IN THIS ISSUE

- 1 Chromatin Immunoprecipitation using General Transcription Factors
- 1 Colorimetric Quantitation of MAPK-regulated Transcription Factors
- 2 Higher Specificity for More Effective Gene Silencing
- 3 Simple, Specific Detection of Epidermal Growth Factor Receptor Phosphorylation
- 6 Quantitative Measurement of Ku and G/T Binding Protein
- 7 TimeLogic Delivers Affordable, Genome-scale BLAST Solutions
- 8 Measure NO Production Faster and More Accurately

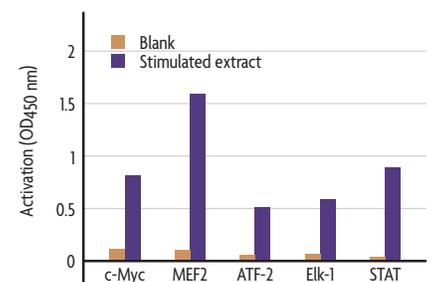


Figure 1: TransAM profiling MAPK-regulated transcription factors.
The TransAM MAPK Family Kit was used to assay the activation levels of c-Myc, MEF2, ATF-2, Elk-1 and STAT using 5 µg/well nuclear extract prepared from either HeLa (TPA treated), Jurkat (unstimulated) or U-937 (TPA + IFN γ treated) cells.

continued on page 4

Higher Specificity for More Effective Gene Silencing

Active Motif's custom gripNA™ oligonucleotides bind to nucleic acids with higher affinity and more stringent mismatch discrimination than other gene silencing reagents. This improves the results of your gene silencing experiments by minimizing non-specific interactions that can cause unintended, mutant phenotypes. gripNAs have proven to be effective at silencing genes in zebrafish, mammalian cells, *Xenopus* and in *in vitro* translation systems. Because gripNAs are resistant to nuclease degradation, they are stable in the *in vivo* environment, enabling them to silence genes expressed over a range of developmental stages. And, you can deliver gripNAs into cells using Chariot™ II, lipids, electroporation, microinjection and the scrape method, making gripNAs the most versatile tool available for gene silencing.

Enhanced PNA chemistry

gripNAs are a negatively charged form of Peptide Nucleic Acids (PNAs). PNAs are DNA analogs in which the nucleoside bases are attached to a synthetic backbone rather than to deoxyribose, as in DNA. While PNAs bind complementary DNA and RNA by conventional Watson-Crick base pairing, they hybridize with greater affinity and increased sequence specificity. Their binding affinity is significantly reduced (or completely eliminated) if there is even a single base-pair mismatch with their target nucleic acid (Table 1). This minimizes the non-specific silencing of genes with sequences that are nearly identical to your target sequence.

Why choose gripNAs?

- Unsurpassed sequence specificity
- Resistant to nucleases
- Flexible synthesis modifications
- Multiple delivery options
- Simple online ordering

	DNA/DNA		gripNA/DNA		Sequence of DNA oligonucleotide
	T _m (°C)	ΔT _m (°C)	T _m (°C)	ΔT _m (°C)	
No mismatches	63.9	–	58.6	–	5'-CAC-TGA-CTT-GAG-ACC-A-3'
Mismatch A	57.0	6.9	41.3	17.3	5'-CAC-TGA- <u>GTT</u> -GAG-ACC-A-3'
Mismatch B	49.7	14.2	No T _m	–	5'-CAC-TGA- <u>GTG</u> -GAG-ACC-A-3'
Mismatch C	61.8	3.1	54.6	4.0	5'-CAC-TGA-CTT-GAG- <u>ACG</u> -A-3'
Mismatch D	56.7	7.2	No T _m	–	5'- <u>CGG</u> -TGA-CTT-GAG-ACC-A-3'
Mismatch E	53.6	10.3	No T _m	–	5'-CAC-TGA- <u>CGT</u> -GAG-ACC-A-3'
Mismatch F	56.2	7.1	No T _m	–	5'-CAC-TGA- <u>CTG</u> -GAG-ACC-A-3'
Mismatch G	54.2	9.7	42.4	16.2	5'-CAC-TGA- <u>CAT</u> -GAG-ACC-A-3'

Table 1: Increased binding specificity of gripNAs.

Identical 16-mer gripNA and DNA probes were synthesized with the sequence 5'-TGG-TCT-CAA-GTC-AGT-G-3'. These were annealed to a complementary DNA oligo (5'-CAC-TGA-CTT-GAG-ACC-A-3') in Hybridization Buffer (20 mM Tris, pH 7.5, 500 mM NaCl, 10 mM MgCl). Each sample was heated to 90°C for 3 minutes, then cooled gradually to room temperature. The samples were then heated at a rate of 1°C per minute from 20°C to 100°C using a thermal control unit linked to a spectrophotometer. Changes in A₂₆₀ were recorded and a melting temperature (T_m) was calculated for the DNA/DNA and gripNA/DNA duplexes. The experiment was then repeated by hybridizing the gripNA and DNA probes to a series of DNA oligos containing one- or two-base mismatches (shown above, with the mismatches underlined). T_m values were measured for each DNA/DNA and gripNA/DNA duplex and a ΔT_m was calculated by subtracting the difference in the melting temperatures of the complementary and mismatched probes. Samples that are unable to form a stable duplex generate a "No T_m" value in this assay and no ΔT_m.

Hit your target only

A recurring problem of many gene silencing reagents is a lack of specificity. This can cause unintended phenotypes because these reagents may bind and silence expression of more than just the intended target. *In vivo* mismatch discrimination experiments have shown that gripNA binding is reduced or eliminated by the presence of a single base-pair mismatch. Recent experiments performed in zebrafish embryos indicate that this specificity can improve your results. While wild-type gripNA and morpholino probes were comparable in potency at silencing the *chordin*, *uroD* and *no tail* genes, the intentional inclusion of 2 and 4 base-pair mismatches in the morpholino probes caused non-specific effects that were not caused by comparable gripNA probes.¹

Order anytime

Active Motif's custom gripNA synthesis service can provide you with better materials for your gene silencing experiments. All the information needed to design, order and use gripNA probes can be accessed at www.activemotif.com/gripna. Our simple, online ordering system makes it easy to place your gripNA order 24 hours a day, 7 days a week. gripNA probes can be ordered with or without Chariot II. In addition, you can add a 3' modification (Biotin, FITC or primary amine) to your probe. Each gripNA is verified by mass spectrophotometry and supplied with a fluorescently labeled positive control for human CREB. Log on now and order your custom gripNA today.

1. Urtishak et al. (2003) *Developmental Dynamics* 228(3).

Product	Format	Catalog No.
Custom gripNA™ Probe	200 nmol	24001
Custom gripNA™ Probe w/Chariot™ II	200 nmol	24002
Primary Amine Modification		24004
Biotin Modification		24005
Fluorescein Modification		24006
Chariot™ II	96 rxns	24008
gripNA™ Human CREB Positive Control	25 nmol	24007
gripNA™ Chordin Positive Control	5 nmol	24009

Simple, Specific Detection of Epidermal Growth Factor Receptor Phosphorylation

Fast Activated Cell-based ELISA (FACE™) Kits are an innovative alternative to classical methods for monitoring protein phosphorylation. FACE Kits enable modification-specific analysis directly within the cell without the need for time-consuming cell extractions, gel electrophoresis or membrane blotting. FACE Kits are designed to overcome the limitations of classical assays and at the same time improve the quality of your results.

Regulation of epidermal growth factor receptor (EGFR) activity via phosphorylation of tyrosine 992 has been shown to be critical for normal functioning of this key cellular receptor. Indeed, important disease states such as cancer have been attributed to aberrant control of Y992 phosphorylation. This, and its cell-surface location, has made EGFR a promising target for anti-cancer therapeutics.

The FACE method

In the FACE method, cells are cultured in 96-well plates and stimulated to induce the pathway of interest. Following stimulation, the cells are fixed, which preserves protein modifications, including phosphorylation. Each well is then incubated with a primary antibody specific for the activated protein of interest. Subsequent incubation with secondary HRP-conjugated antibody and developing solution provides an easily quantified, colorimetric or chemiluminescent readout. The signals are then normalized for cell number using the provided Crystal Violet solution and plotted. FACE Kits are easy to use and require less than two hours hands on time, giving you convenience not available with other phosphorylation assays.

Specific antibodies

In order to get the most accurate results possible, it is essential that antibodies which bind only to the protein or protein modification of interest be used in detection. This is even more important when using ELISA-based techniques that do not

involve the visualization of protein bands. All antibodies included in FACE Kits are extensively tested and compared to Western analysis to ensure your analysis is the most accurate and sensitive possible (Figure 1).



Figure 1: Western analysis of phosphorylated EGFR.

The phospho-EGFR antibody contained within FACE EGFR Kits was used in Western analysis to verify antibody specificity. (Multiple bands present in lanes 1 and 2 are due to EGF glycosylation).

Lane 1: 8 µg whole-cell extract from EGF-induced A-431 cells.
Lane 2: 8 µg whole-cell extract from non-induced A-431 cells.

Sensitivity without compromise

Achieving the sensitivity required to detect some low-abundance kinases and kinase-regulated proteins can be challenging. In the past, a lack of sensitivity has typically meant that scientists must either load more of each sample, or change the cellular model. Both of these solutions are far from optimal. The improved sensitivity of FACE compared to standard techniques, means that you

won't have to make such compromises ever again. To demonstrate the sensitivity achieved when using the colorimetric FACE Kits, levels of phosphorylated EGFR present in HeLa cells stimulated using EGF were measured and compared to an equivalent Western blot. Despite loading significant amounts of sample (20 µg per well) and prolonged development, detection of phosphorylated EGFR by Western blot was not possible. In contrast, detection of phosphorylated EGFR using FACE was simple and quantitative (Figure 2). And, for those who require even more sensitivity, all FACE Kits are also available in an ultra-sensitive chemiluminescent format. Don't compromise, when you can use FACE Kits today.

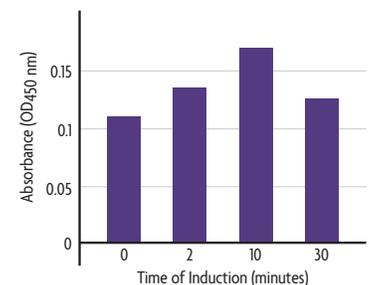


Figure 2: Detection of EGFR phosphorylation using FACE.

The FACE EGFR Kit was used to assay the level of phosphorylated EGFR contained within EGF stimulated HeLa cells. FACE assays were performed before and 2, 10 and 30 minutes after EGF induction.

Product	Format	Catalog No.
FACE™ AKT	1 x 96 rxns	48120
	5 x 96 rxns	48620
FACE™ AKT Chemi	1 x 96 rxns	48220
	5 x 96 rxns	48720
FACE™ EGFR	1 x 96 rxns	48150
	5 x 96 rxns	48650
FACE™ EGFR Chemi	1 x 96 rxns	48250
	5 x 96 rxns	48750
FACE™ ERK1/2	1 x 96 rxns	48140
	5 x 96 rxns	48640
FACE™ ERK1/2 Chemi	1 x 96 rxns	48240
	5 x 96 rxns	48740
FACE™ JNK	1 x 96 rxns	48110
	5 x 96 rxns	48610
FACE™ JNK Chemi	1 x 96 rxns	48210
	5 x 96 rxns	48710
FACE™ p38	1 x 96 rxns	48100
	5 x 96 rxns	48600
FACE™ p38 Chemi	1 x 96 rxns	48200
	5 x 96 rxns	48700

continued from page 1 — Colorimetric Quantitation of MAPK-regulated Transcription Factors

The TransAM method

TransAM MAPK Family Kits utilize a 96-well plate, with each well containing six different double-stranded oligonucleotides. When nuclear or whole-cell extract is added, the various MAPK-regulated transcription factors bind to the consensus-binding site on their particular oligonucleotide. Primary antibodies specific for the activated forms of each transcription factor are then added to individual wells, followed by incubation with secondary HRP-conjugated antibody and developing reagent. The plate is then read on a spectrophotometer, which provides a quantitative, colorimetric readout (Figure 2). Thus, TransAM MAPK Family Kit makes it possible to rapidly profile the levels of each MAPK-regulated transcription factor under various growth and treatment conditions.

Improved method

TransAM assays are a marked improvement over other techniques used to study transcription factor activation. Unlike gelshift, Western blotting and reporter gene methods, TransAM does not use inefficient cloning and cell transfections, time-consuming gel exposures or radioactive probes. Inconsistencies due to variable reporter plasmid transfection and the need to construct stable cell lines are also eliminated. TransAM assays are complete in mere hours, rather than in days, are more sensitive and provide quantitative results. Moreover, TransAM can be used to study stimulated tissue samples.

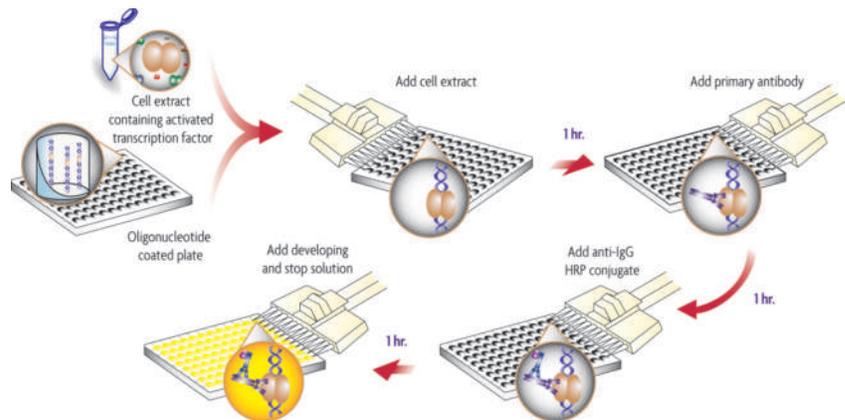


Figure 2: Flowchart of the TransAM procedure.

TransAM advantages

- Non-radioactive, colorimetric method provides quantifiable results
- Results in less than 5 hours
- 10-fold greater sensitivity than gelshift
- Simultaneous profiling of multiple family members
- Ability to assay cell and tissue samples

The TransAM Family

In addition to the new TransAM MAPK Family Kit, Active Motif offers TransAM Family Kits for simultaneously studying members of the AP-1, GATA, HNF, NFκB, IRF and STAT transcription factor families. TransAM Kits for monitoring the individual transcription factors NFκB** p50 and p65, HIF-1, c-Fos, FosB, c-Jun, PPARγ, p53, MyoD, Sp1, Sp3, C/EBP, NF-YA, ER, STAT3, c-Myc, Elk-1,

NFATc1, and total and phosphorylated CREB are also available. Ultra-sensitive TransAM Chemiluminescent Kits are also available for NFκB p50 and p65. For a more convenient and better method to study transcription factor activation, try TransAM Kits.

New TransAM Kits for c-Myc & Elk-1

In addition to the new TransAM MAPK Family Kit, Active Motif is introducing two individual TransAM Kits for c-Myc and Elk-1. Because these two transcription factors are directly related to MAPK cascades, they are widely studied. The new TransAM Kits enable users to study the activation of c-Myc and Elk-1 individually.

* Technology covered under AAT-filed patents and licensed exclusively to Active Motif.

** Use of TransAM in NFκB-related drug discovery may be covered under U.S. Patent No. 6,150,090 and require a license from Ariad Pharmaceuticals (Cambridge, MA, USA).

Product	Format	Catalog No.
TransAM™ MAPK Family	1 x 96-well plate	47296
TransAM™ c-Myc	1 x 96-well plate 5 x 96-well plates	43396 43896
TransAM™ Elk-1	1 x 96-well plate 5 x 96-well plates	44396 44896

continued from page 1 — Chromatin Immunoprecipitation using General Transcription Factors

Transcriptional regulation is one of the main methods used by cells to control gene expression. Traditionally researchers have analyzed transcription factors and gene promoter activity using methods such as reporter gene assays, EMSA, Western blotting and more recently, using DNA microarrays. However, although these methods have led to significant advances in the scientific understanding of transcription, there is still a need for improved assays. Specifically, there is a need for better understanding of *in vivo* interactions between DNA and transcription factors. The ChIP method overcomes this limitation through a formaldehyde cross-linking step, which “freezes” and preserves cellular protein/DNA interactions.

The complete solution

Classically, ChIP has been performed using antibodies directed against abundant chromatin components, such as acetylated histones. However, while this method can indicate the transcriptional activity of a given promoter, it does not reveal the specific transcription factor bound to the promoter(s) of interest. In contrast, ChIP performed using transcription factor-specific antibodies enables the direct monitoring of transcription factor/DNA interactions. However, this method is technically more challenging and requires the preparation of several complicated buffers, inhibitors and blocking reagents. Preparation of these individual components within the lab can be a time consuming and expensive undertaking. Unlike other commercially available ChIP kits, Active Motif's ChIP-IT Kit provides you with all the reagents and controls needed to successfully perform your ChIP experiments. Plus, the ChIP-IT Kit also contains positive control antibody directed against the general transcription factor, transcription factor IIB (TFIIB). Because TFIIB binds to nearly all active promoters, it is an ideal positive control if you are trying to validate other transcription factor-specific antibodies. No other kit available today can provide you with such a complete solution.

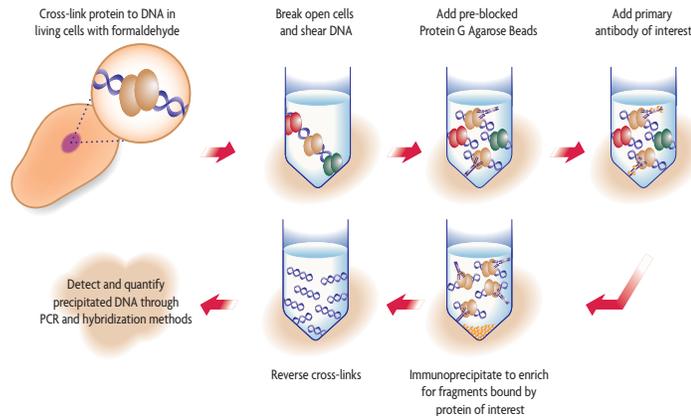


Figure 1: Schematic of Chromatin Immunoprecipitation.

The ChIP method

In the ChIP-IT method, intact cells are fixed using formaldehyde, which cross-links and therefore preserves protein/DNA interactions. DNA is then sonicated into small uniform fragments and the DNA/protein complexes are immunoprecipitated using an antibody directed against the DNA-binding protein of interest. Following immunoprecipitation, cross-linking is reversed and the DNA is screened to determine which genes, or groups of genes, were bound by the protein of interest (Figure 1). The versatility of ChIP means that screening can be done using generalized hybridization or a more targeted PCR-based approach, depending on the needs of your experiment.

Proven effective

Successful ChIP depends on sonicated DNA of the correct length and antibody(ies) that are effective at immunoprecipitating protein/DNA complexes. However, it can be difficult to determine whether “pull down” was successful or not, unless you possess an antibody that has already been proven effective in ChIP. So that time spent

on antibody validation is minimized, the TFIIB antibody included in the ChIP-IT Kit has been rigorously tested and proven to “pull down” TFIIB-bound DNA. And, because TFIIB binds almost all active promoters, you can be certain that if your DNA is suitable for ChIP, it will work with the ChIP-IT Kit. Using the ChIP-IT Kit there will be no need to waste time and money wondering whether your ChIP has worked or not.

Controls for confidence

Specificity of antibody binding is essential for getting the most accurate data from your ChIP experiments. It is important that any non-specific DNA precipitation, which can result in incorrect analyses, is easily identified. As such, the ChIP-IT Kit contains several PCR primers, which are directed against DNA known to either bind TFIIB or not. And, because the TFIIB antibody is known to be highly specific in ChIP you can analyze your DNA to ensure “pull-down” was specific. Collectively, these proven reagents will help ensure the validity of your results. To get the most out of your ChIP experiments, buy the ChIP-IT Kit today.

Product

ChIP-IT Kit

Catalog No.

53001

Quantitative Measurement of Ku and G/T Binding Protein

Active Motif's new DNA Repair Kits utilize a novel method* that makes the study of DNA repair proteins faster and more sensitive. Unlike conventional techniques such as EMSA and Western blot, these kits are DNA-binding ELISAs that provide quantitative results in less than five hours, while eliminating the use of radioactivity. Studying DNA repair has never been so simple.

Ku and G/T binding protein (GTBP) are damage recognition proteins that bind as heterodimers to DNA with specific lesions. Once bound, these proteins catalyze repair of the DNA and have been shown to be critical in maintaining genome integrity. Despite the interest in studying DNA damage and repair, there is a lack of convenient, sensitive assays that are suitable for modern high-throughput research. That's why we have developed the new Ku70/86 and GTBP DNA Repair Kits.

Advantages

- Non-radioactive, colorimetric method
- 10X more sensitive than EMSA
- Results in under 5 hours
- Works with cells and tissues
- High-throughput compatible

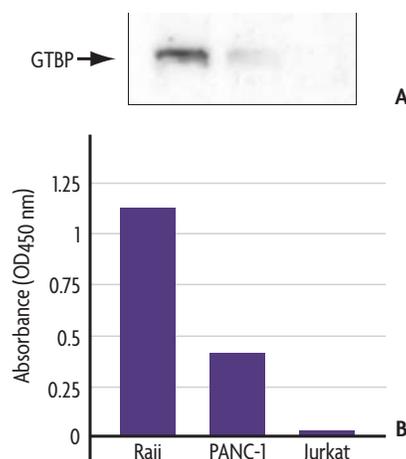


Figure 2: Measurement of GTBP activity in different cell lines using Western blot and the new GTBP DNA Repair Kit. Raji, PANC-1 and Jurkat nuclear extracts were assessed for GTBP activity using Western blot (A) and the new GTBP DNA Repair Kit (B).

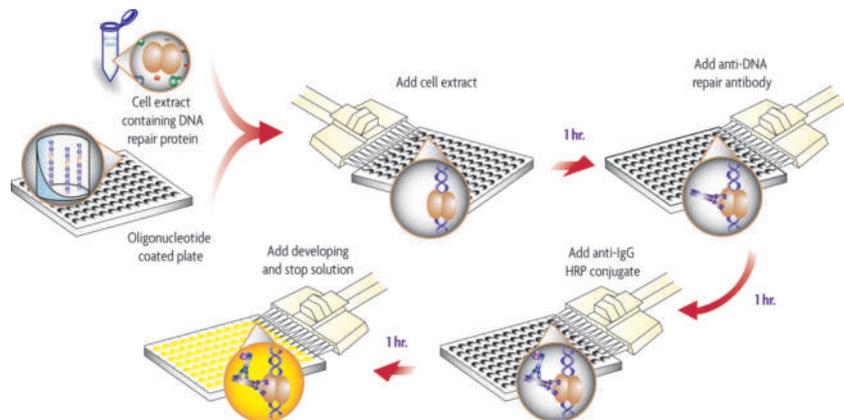


Figure 1: Flowchart of the DNA Repair Kit procedure.

Simple, efficient method

The DNA Repair Kit method is simple and efficient and can be used with both tissue and cell culture extracts. Each kit includes a 96-well plate in which multiple copies of a specific double-stranded oligonucleotide have been immobilized. The oligonucleotide in the GTBP Kit contains a G/T mismatch, while that in the Ku70/86 Kit simply has a blunt end. When cellular extract is added, the repair protein of interest binds to the oligonucleotide. Each well is then incubated with a primary antibody that is specific for the repair protein being studied. Addition of a secondary HRP-conjugated antibody and developing solution provides an easily quantified colorimetric readout (Figure 1). Now you can efficiently monitor the regulation of DNA repair proteins under different treatments.

Easily quantitate your data

Being able to measure small changes in DNA repair protein activity is important. While traditional methods such as Western

blot are sensitive, they typically provide qualitative, rather than quantitative data. Active Motif's DNA Repair Kits, however, provide a colorimetric readout that is easily quantified, so you can detect even the slightest change in protein activity. To illustrate, GTBP activity was measured and compared using Active Motif's GTBP DNA Repair Kit and by Western blot. The GTBP DNA Repair Kit results are clearly more quantitative than Western blot (Figure 2).

Simplify your detection

Active Motif's new Ku and GTBP DNA Repair Kits will simplify the way you study interactions between damaged DNA and repair proteins. These easy-to-use kits are flexible enough to use in a variety of functional studies, such as examining the specificity of repair proteins for a particular DNA damage. For a better method to study DNA repair protein activity, try one of Active Motif's new DNA Repair Kits.

* Patent Pending.

Product	Format	Catalog No.
GTBP DNA Repair Kit	1 x 96-well plate	51096
	5 x 96-well plates	51596
Ku70/86 DNA Repair Kit	1 x 96-well plate	51196
	5 x 96-well plates	51696

TimeLogic® Delivers Affordable, Genome-scale BLAST Solutions

TimeLogic was recently acquired by Active Motif to help researchers connect wet lab science and computational biology. TimeLogic's BLAST System accelerates algorithms critical for genomic analysis, SNP identification and all-to-all database comparisons. It can bring, within a single server, the performance of hundreds of CPUs. With an intuitive web interface and command line access for batch searching, BLAST is a powerful tool for all levels of bioinformatics expertise.

TimeLogic's acceleration technology makes it possible to process extremely large-scale genomic analyses with BLASTN or BLASTP, and also employ rigorous, frame-translated methods for more effective searching of divergent sequences (T-BLASTX).



The BLAST system is driven by DeCypher®, an innovative combination of software and reconfigurable hardware that processes trillions of base comparisons per second. Beyond dramatic BLAST analysis speed, the system offers many advantages:

- **Greater default sensitivity than NCBI BLAST:** Find more hits per search
- **Smaller footprint:** The computing capability of a server farm in the space of a single computer
- **Low ownership costs:** Unlike clusters, DeCypher brings no increased costs for power, cooling and maintenance
- **Simple scalability:** Increase performance as your search needs grow simply by adding additional DeCypher acceleration cards

The TimeLogic BLAST System is affordable for departmental budgets, and installs rapidly within a server from Sun Microsystems or Dell Computers. Please contact us to find out how the TimeLogic BLAST System can accelerate your research goals.

Contact Information

TimeLogic Corporation
U.S. +1 775 833-0200 (PDT)
<http://www.timelogic.com>

TimeLogic®

biocomputing accelerated

Visit our web site to find out about additional TimeLogic BLAST, HMM and Smith-Waterman Systems.

BLAST Comparison of SNP Data with the Human Genome

The aim of pharmacogenomics is to develop drugs that can be specifically targeted to a patient's genotype group for high efficacy response and no side effects. Extensive progress in mapping SNPs, which are single nucleotide polymorphisms occurring in over 1% of a population, has brought rapid advances to this nascent field.

Identifying SNPs involved in novel disease targets, whether in a coding (cSNP) or regulatory (rSNP) capacity, is a key focus. With the help of population-based SNP studies, pharmacogenomic therapies and potential preventative approaches can be designed to address genetic risk factors.

Comparing a large collection of SNP data to monthly Human genome updates can drain an institution's computational resources. However, this is a critical task for research groups pursuing pharmacogenomics-based therapies.

To illustrate the BLAST System's speed and efficiency for pharmacogenomics, population genetics and modern genetic toxicology, TimeLogic recently performed a private, large-scale benchmark. A BLASTN search was used to compare 1.7 million 25-mer nucleic sequences of proprietary SNP data (a total

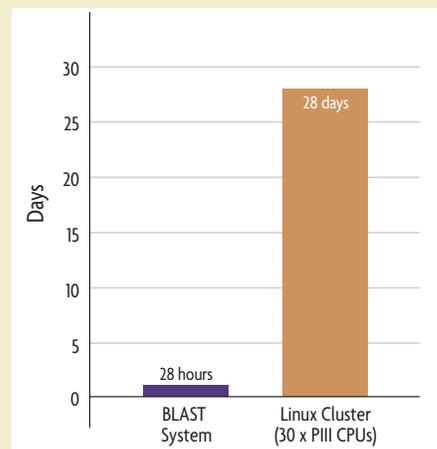


Figure 1: BLAST System vs. a Linux Cluster.
17 million 25-mers compared to the human genome with BLASTN.

of 42,500,000 bases) against the human genome target of 3,099,043,584 bases.

The DeCypher BLAST Solution completed the search in 28 hours, or 24X faster than a Linux cluster of 30 Pentium III 1 GHz CPUs (Figure 1). This test demonstrates performance equivalent to a server farm of over 700 CPUs. For additional details on this benchmark and other performance measures, please visit:

<http://www.timelogic.com/performance/>.

Measure NO Production Faster and More Accurately

Active Motif's Nitric Oxide Quantitation Kit provides a fast and sensitive method for measuring the production of nitric oxide (NO) in your samples. The kit employs an innovative cofactor technology that reduces the time and number of steps needed to measure NO levels. Plus, the Nitric Oxide Quantitation Kit has a wider dynamic range of NO measurement than conventional technology (Figure 1), giving you more accurate results.

Advantages

- Increased sensitivity
- Fast and easy to use
- Wider dynamic range
- High-throughput compatible
- Works with a variety of sample types

The old way has more steps

Typically, NO assays are performed using either a two-step assay or a three-step lactate dehydrogenase (LDH) assay. In both methods, the first step is the reduction of nitrate into nitrite by nitrate reductase. In the final step, Griess Reagent converts

the nitrite into a purple-colored azo compound that is quantitated using a spectrophotometer. However, excess NADPH, an essential cofactor in nitrate reduction, interferes with the Griess reaction, limiting the sensitivity of the two-step assay. To improve sensitivity, an LDH step can be added before the Griess Reagent step. Though this increases the sensitivity of the assay, it is more time-consuming and complicated.

A faster procedure

Our Nitric Oxide Quantitation Kit offers a better alternative than conventional Two- and Three-Step NO assays. The kit contains a unique formulation of cofactors that accelerate the conversion of nitrate to nitrite, while simultaneously degrading NADPH. This decreases the reductase step to just 30 minutes and eliminates the complicated, time-consuming third step.

Get yours today

Active Motif's Nitric Oxide Quantitation Kit makes NO detection quick and easy. Simplify the way you measure NO production and order a Nitric Oxide Quantitation Kit today!

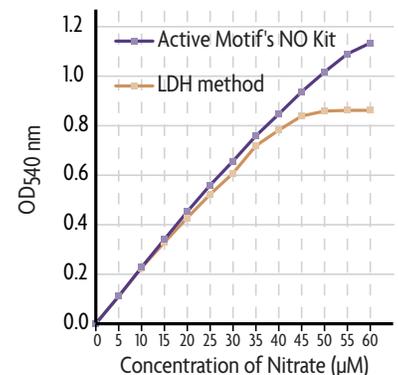


Figure 1: Dynamic range of nitrate standard curves.

Nitrate standard curves produced using the Nitric Oxide Quantitation Kit and a conventional Three-Step LDH-method kit.

Product	Format	Catalog No.
Nitric Oxide Quantitation Kit	2 x 96 rxns	40020

CONTACT INFO

U.S.

1914 Palomar Oaks Way, Suite 150
 Carlsbad, CA 92008
 Toll Free: 877 222 9543
 Direct: 760 431 1263
 Fax: 760 431 1351
 Email: tech_service@activemotif.com

EUROPE

104 Avenue Franklin-Box 25
 B-1330 Rixensart, Belgium
 Toll Free: +800 222 95 430
 Direct: +32 (0)2 653 0001
 Fax: +32 (0)2 653 0050
 Email: eurotech@activemotif.com