Transcription Factor ELISAs

TransAM®

sensitive quantitative transcription factor ELISAs

TransAM® Kits are DNA-binding ELISAs that facilitate the study of transcription factor activation in mammalian tissue and cell culture extracts.

TransAM advantages

- Highly specific assay provides quantitative results in under 5 hours
- Eliminates the use of radioactivity and the need to run gels
- Up to 100-fold more sensitive than gelshift assays
- No cloning or transfection required
- Ability to assay tissue samples

ACTIVE MOTIF®

Tools to Analyze Nuclear Function

TransAM® – sensitive, non-radioactive transcription factor ELISAs

TransAM® Kits are DNA-binding ELISAs* that facilitate the study of transcription factor activation in mammalian tissue and cell culture extracts. The novel TransAM method is up to 100-fold more sensitive than gelshift and is complete in less than 5 hours. TransAM Kits eliminate the use of radioactivity and the need to run gels, while the high-throughput format enables simultaneous screening of 1 to 96 samples in a single experiment. And unlike reporter assays, TransAM can be used on all sample types, including cell lines and tissues, giving you unsurpassed flexibility.

The study of transcription factors

Understanding and quantifying transcription factors is integral to the study of cellular function in relation to differentiation, brain activity, immune response, inflammation, cancer, and much more. Families of closely related homo- and heterodimer complexes that bind to DNA (transcription factors) regulate many of the global signaling pathways and are widely studied as disease targets.

Traditionally, transcription factors have been studied using four methods: gelshift, supershift/Electrophoretic Mobility Shift Assays (EMSA), immunoblotting and reporter gene assays. These methods are time-consuming and at best provide only semi-quantitative results. Moreover, they don't support high-throughput methods and tend to lack both sensitivity and reproducibility.

The TransAM® method

Each TransAM Kit includes a 96-stripwell plate in which multiple copies of a specific double-stranded oligonucleotide have been immobilized. When nuclear or whole-cell extract is added, the transcription factor of interest binds the oligonucleotide at its consensus binding site. A primary antibody directed against the transcription factor of interest is added, followed by a secondary HRP-conjugated antibody and HRP substrate (Figure 1). The colorimetric change is measured with a spectrophotometer and is directly proportional to the quantity of transcription factor present, providing a sensitive, quantitative assay for transcription factor activation.

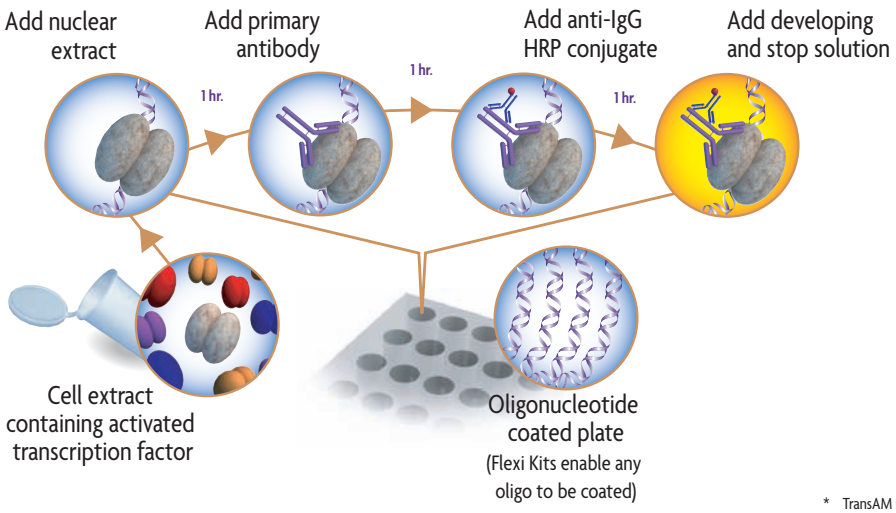

Figure 1: Flow chart of the TransAM procedure.

TransAM® advantages

TransAM Kits are highly sensitive DNA-binding ELISAs capable of detecting small changes in transcription factor levels without the need for gels or radioactivity. TransAM offers the convenience of a fast, quantitative and highly specific assay, making it the most published alternative to EMSA (Figure 2).

- **Sensitive** – 100-fold more sensitive than a gelshift assay
- **Quantitative** – obtain quantitative results in less than 5 hours
- **Non-radioactive** – colorimetric and chemiluminescent formats available
- **Controls ensure success** – includes positive control extract and competitive oligonucleotides
- **Versatile** – over 40 different transcription factor targets

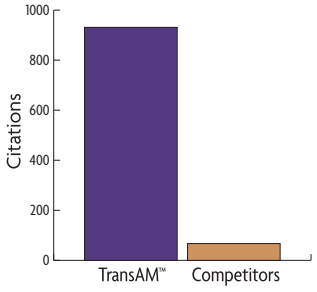

Figure 2: TransAM Kit citations.
Using HighWire Press, http://highwire.standford.edu, a comparison of citations for TransAM from Active Motif versus the tradenames of all competitor kits combined was run. TransAM is clearly the leader.

* TransAM is licensed from EAT under issued and pending worldwide patents. Purchase includes the right to use for basic research only. Other-use licenses available, please contact Technical Services.

The 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).

Sensitive – for improved transcription factor monitoring

Small changes in transcription factor levels can have a significant impact on cellular function. Therefore, it is vital to use a sensitive assay when studying transcription factor activation. TransAM Kits are 10-fold more sensitive than gelshift assays (Figure 3). And, TransAM Chemi Kits are even better, with more than 100-fold increased sensitivity as compared to gelshift assays. Not only does this enable you to monitor small changes in activated transcription factor levels, but you can subtract the level of endogenous factor present in your untreated samples, further improving your results.

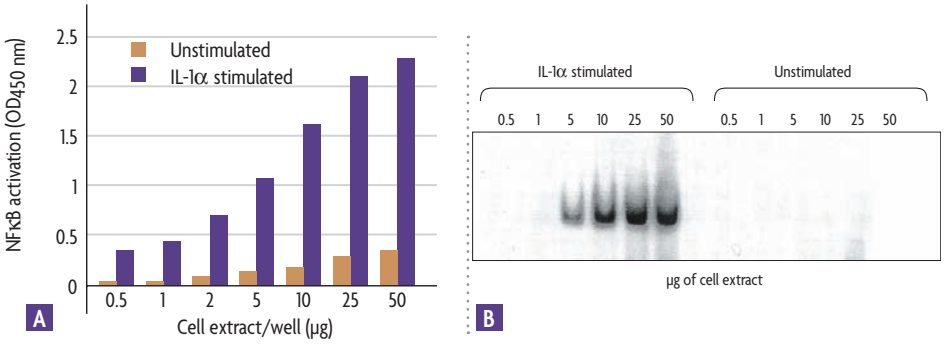

Figure 3: TransAM NFκB is more sensitive than gelshift.
Human fibroblast WI-38 cells are stimulated with IL-1α for 30 minutes. Increasing amounts of whole-cell extract are assayed using the TransAM NFκB p50 Kit (A) or gel retardation (B).

Fast, quantitative results

Western blotting and reporter assays commonly require long incubation periods either following electrophoresis or DNA transfection, which can be frustrating and inconvenient to perform. With TransAM Kits you can go from sample to quantitative results in under 5 hours. Why waste time waiting when you can have your results in the same day?

Easy and safe – non-radioactive, colorimetric assay

Gelshift/EMSA assays utilize the DNA binding capability of transcription factors to bind to radiolabeled oligonucleotide probes prior to electrophoresis. This can be time consuming and hazardous work to perform. While TransAM Kits also utilize the DNA binding properties of transcription factors, unlike traditional methods, they detect activated protein using antibodies rather than radioactivity. This means that you can safely assay for a specific transcription factor without the need for radioactivity or the hassle of running gels. The colorimetric change is easily measured using a spectrophotometer, further improving the ease of using TransAM Kits.

Proven specificity – for improved accuracy

To accurately study transcription factor activation, it is vital to be able to determine which isoform of the transcription factor of interest is involved in pathway regulation. TransAM Kits are tested for specificity by assaying in the presence of an excess of oligonucleotide containing a wild-type or mutated consensus binding site (Figure 4). This competitive assay proves that the transcription factor detected is binding specifically to the probe that has been immobilized to the TransAM plate. Our antibodies are also assayed for cross-reactivity with other closely related family members to ensure that you are detecting the isoform of interest. With TransAM Kits you are guaranteed to detect only the transcription factor that you want.

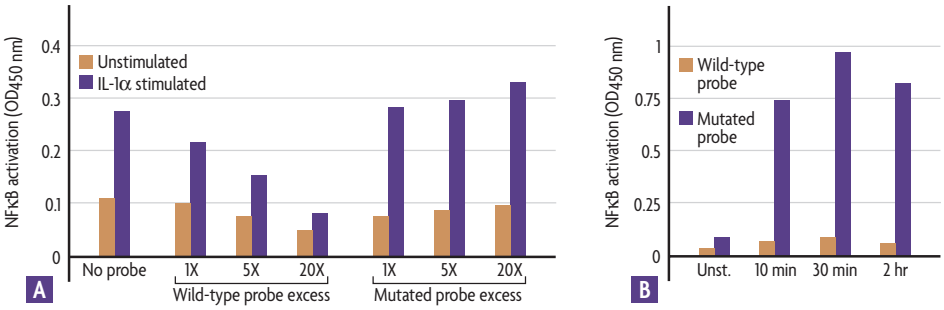

Figure 4: Specificity of TransAM Kits.
TransAM NFκB p50 assays are performed in the presence of wild-type and mutated competitor oligonucleotides using 10 µg/well whole-cell extract from human fibroblast WI-38 cells stimulated with IL-1α for 30 minutes (A) and 5 µg/well whole-cell extract from HeLa cells stimulated with TNF-α for 10 and 30 minutes, and 2 hours (B).

TransAM® Family Kits – NFκB, AP-1, STAT & GATA

Transcription factor families consist of various groups of homo- and heterodimer subunits that are differentially regulated within the cell. Therefore, being able to profile which members of a transcription factor family are involved in cell regulation can help identify potential disease targets. However, profiling an entire transcription factor family using gelshift, Westerns or reporter assays is time consuming and expensive. TransAM Family Kits enable you to profile activation of various transcription factor family members in less than 5 hours using one simple kit. The TransAM NFκB Family Kit includes antibodies specific for detection of activated p65, p50, p52, c-Rel and RelB (Figure 5). The TransAM AP-1 Family Kit includes antibodies specific for active c-Fos, FosB, Fra-1, c-Jun, JunB and JunD. The TransAM STAT Family Kit detects phosphorylated STAT1α, STAT3, STAT5A and STAT5B. The TransAM GATA Family Kit detects GATA-1, GATA-2 and GATA-3. For details, please visit our website at www.activemotif.com/transam.

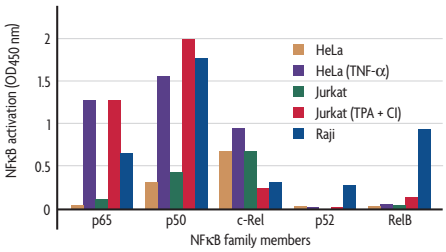

Figure 5: Profiling NFκB family DNA binding activation. Nuclear extracts prepared using the Nuclear Extract Kit from HeLa cells, HeLa cells treated with TNF-α, Jurkat cells, Jurkat cells treated with TPA + calcium ionophore and Raji cells were assayed at 10 µg/well using the TransAM NFκB Family Kit.

TransAM® Multiple-oligo Family Kits – MAPK & HNF

Some transcription families have members that do not share a single consensus-binding site, making EMSA and reporter assays difficult. To overcome this, Active Motif has developed TransAM Family Kits that have multiple binding sites in each well. TransAM HNF Family Kits contain a mixture of oligonucleotides with the HNF-1, -3 and -4 binding sites. HNF dimers contained in nuclear extracts bind specifically to these oligonucleotides and are detected using antibodies against HNF-1, -3α, -3β and -4α (Figure 6). Similarly, the TransAM MAPK Family Kit contains a mixture of oligos and antibodies that enable detection of activated ATF-2, c-Jun, c-Myc, MEF2 and STAT1α. By immobilizing a mixture of binding sites, each well in these kits can be used to assay any family member.

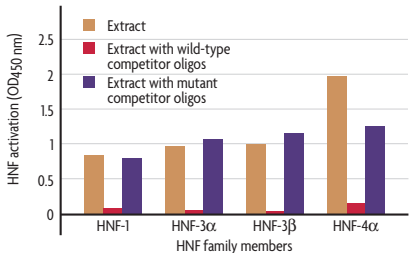

Figure 6: Specific assay for HNF family members. Nuclear extracts from unstimulated Hep G2 cells were assayed at 5 µg/well using the TransAM HNF Family Kit in the absence or presence of 20 pmol of a mixture of competitor oligonucleotides.

TransAM® Flexi Kits

TransAM Flexi Kits enable efficient study of transcription factor binding at any DNA-binding site. With TransAM Flexi, you can study variant transcription factor-binding sites, analyze native promoters (Figure 8), confirm chromatin immunoprecipitation (ChIP) results and determine isoform-binding affinity. To measure your site of interest, first design appropriate biotinylated oligos. Each oligo is incubated with nuclear extract and transferred to a 96-well streptavidin-coated plate. Primary antibody for the factor of interest is added, followed by secondary antibody and developing reagent.

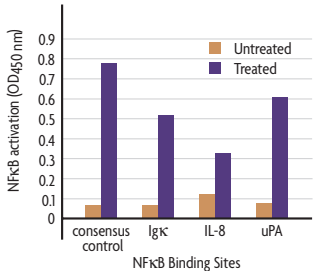

Figure 8: Testing natural NFκB binding sites. Five µg of nuclear extract from untreated and TNF-α-treated HeLa cells were used to assay for NFκB binding affinity to four different 50-mer oligonucleotides. Three oligos were synthesized to represent the natural binding sites on various promoters regulated by NFκB: Igκ, IL-8 and uPA.

TransAM® Chemi Kits

Researchers who require maximum sensitivity or with a limited amount of sample will love TransAM Chemi Kits. With chemiluminescent detection, transcription factor activation can be assayed using as little as 40 ng of cell extract (Figure 7). This makes TransAM Chemi Kits over 100-fold more sensitive than gelshift assays. Plus, you get the added convenience of TransAM's non-radioactive, 96-stripwell format. Don't waste your precious sample or be forced to pool together multiple samples; assay your samples with TransAM Chemi Kits.

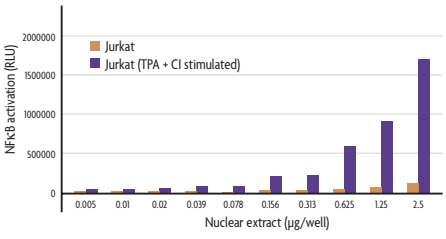

Figure 7: TransAM NFκB p50 Chemi sensitivity. Nuclear extracts from Jurkat cells and Jurkat cells stimulated with TPA + calcium ionophore were assayed from 0.005 to 2.5 µg/well for NFκB p50 activation using the TransAM NFκB p50 Chemi Kit.

Recombinant protein standard curves

In addition to the TransAM transcription factor DNA-binding ELISAs, Active Motif also sells an extensive line of recombinant proteins including transcription factors and cell-signaling-related proteins. These recombinant proteins are ideal for all your research needs and can be used in a variety of applications, including as protein standards in ELISAs. The c-Fos, c-Jun, c-Myc, CREB, NFκB p50, NFκB p65, p53 and Sp1 proteins have been validated for use in making standard curves in our TransAM Kits (Figure 9).

Product	Format	Cat. No.
Recombinant c-Fos protein	5 µg	31115
Recombinant c-Jun protein	5 µg	31116
Recombinant c-Myc protein	5 µg	31117
Recombinant NFκB p50 protein	5 µg	31101
Recombinant NFκB p65 protein	5 µg	31102
Recombinant p53 protein	5 µg	31103
Recombinant Sp1 protein	2 µg	31137

To see all of our recombinant proteins, go to www.activemotif.com/proteins.

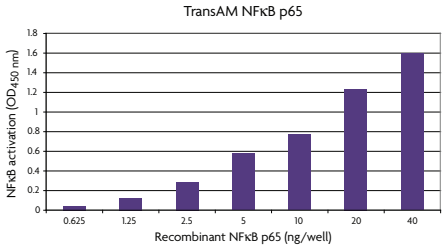

Figure 9: Recombinant protein standards in TransAM. Recombinant NFκB p65 was assayed using the TransAM NFκB p65 kit from 0.625 - 40 ng/well. The standard curve generated from the recombinant protein can be used to quantify the amount of transcription factor in a given sample.

Nuclear Extract Kit – simplifies sample preparation

Active Motif's Nuclear Extract Kit is ideal for the preparation of nuclear, whole-cell and cytoplasmic extracts from mammalian cells and tissues. The resultant high-quality extracts are compatible for use with the TransAM transcription factor ELISAs and other assays. The Nuclear Extract Kit eliminates the need to optimize reagents and ensures consistently high yields. The detailed protocol helps ensure that your extract is not contaminated with proteins from other cellular compartments (Figure 10).

In the Nuclear Extract Kit, cells are collected in ice-cold PBS in the presence of phosphatase inhibitors to limit further protein modifications. Next, the cells are resuspended in hypotonic buffer to swell the cell membrane. Addition of detergent causes leakage of the cytoplasmic proteins into the supernatant. After collection of the cytoplasmic fraction, the nuclei are lysed and the nuclear proteins are solubilized in lysis buffer in the presence of protease inhibitors. Whole-cell extracts can also be prepared by collecting the cells in the PBS/phosphatase inhibitor solution and lysing in lysis buffer. Solubilized proteins are separated from the cell debris by centrifugation.

Product	Format	Cat. No.
Nuclear Extract Kit	100 rxns	40010
	400 rxns	40410

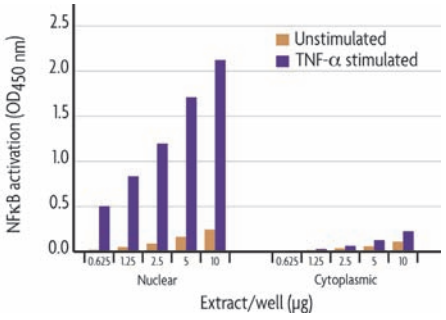

Figure 10: Nuclear Extract Kit provides specific extraction of nuclear and cytoplasmic proteins. Nuclear and cytoplasmic extracts were prepared using the Nuclear Extract Kit from HeLa samples unstimulated or stimulated with TNF-α for 30 minutes and assayed using the TransAM NFκB p50 Kit. Because activated NFκB translocates to the nucleus, only nuclear extract from stimulated cells should contain activated NFκB.

Latest additions

We are continually developing new TransAM Kits. Plus, we also now offer functionally active recombinant proteins. To learn more, please go to www.activemotif.com/transam for an up-to-date list of available kits and to view kit-specific information, download product manuals, *etc*.

Contents & storage

The original format of TransAM provides the oligonucleotide precoated to the wells of the plate. The Flexi format of TransAM requires the user to synthesize their own oligos. All TransAM Kit formats provide one, two or five 96-stripwell assay plate(s) with plate sealer(s), primary antibody(ies), HRP-conjugated secondary antibody, wild-type and mutated oligonucleotides, positive control cell extract, Protease Inhibitor Cocktail, Lysis and Reaction Buffers. Storage conditions vary from room temperature to -80°C. All reagents are guaranteed stable for 6 months when stored properly.

TransAM® – Ordering information

Factor	Product	Format	Cat. No.
AML/Runx	TransAM® AML-1/Runx1	1 x 96-well plate	47396
		5 x 96-well plates	47896
	TransAM® AML-3/Runx2	1 x 96-well plate	44496
		5 x 96-well plates	44996
AP-1	TransAM® AP-1 Family[1]	2 x 96-well plates	44296
	TransAM® AP-1 c-Fos	1 x 96-well plate	44096
		5 x 96-well plates	44596
	TransAM® AP-1 c-Jun	1 x 96-well plate	46096
		5 x 96-well plates	46596
	TransAM® AP-1 FosB	1 x 96-well plate	45096
		5 x 96-well plates	45596
	TransAM® AP-1 JunD	1 x 96-well plate	43496
		5 x 96-well plates	43996
ATF-2	TransAM® ATF-2	1 x 96-well plate	42396
		5 x 96-well plates	42896
c-Myc	TransAM® c-Myc	1 x 96-well plate	43396
		5 x 96-well plates	43896
C/EBP	TransAM® C/EBP α/β	1 x 96-well plate	44196
		5 x 96-well plates	44696
CREB	TransAM® CREB	1 x 96-well plate	42096
		5 x 96-well plates	42596
	TransAM® pCREB	1 x 96-well plate	43096
		5 x 96-well plates	43596
Elk-1	TransAM® Elk-1	1 x 96-well plate	44396
		5 x 96-well plates	44896
ER	TransAM® ER	1 x 96-well plate	41396
		5 x 96-well plates	41996
FKHR	TransAM® FKHR (FOXO1)	1 x 96-well plate	46396
		5 x 96-well plates	46896
GATA	TransAM® GATA Family[2]	2 x 96-well plates	48296
	TransAM® GATA-4	1 x 96-well plate	46496
		5 x 96-well plates	46996
GR	TransAM® GR	1 x 96-well plate	45496
		5 x 96-well plates	45996
HIF	TransAM® HIF-1	1 x 96-well plate	47096
		5 x 96-well plates	47596
HNF	TransAM® HNF Family[3]	2 x 96-well plates	46296
	TransAM® HNF-1	1 x 96-well plate	46196
		5 x 96-well plates	46696
IRF-3	TransAM® IRF-3 (Human)	1 x 96-well plate	48396
		5 x 96-well plates	48896
	TransAM® IRF-3 (Mouse)	1 x 96-well plate	48496
		5 x 96-well plates	48996
IRF-7	TransAM® IRF-7	1 x 96-well plate	50196
		5 x 96-well plates	50696
MAPK	TransAM® MAPK Family[4]	2 x 96-well plates	47296

Factor	Product	Format	Cat. No.
MEF2	TransAM® MEF2	1 x 96-well plate	43196
		5 x 96-well plates	43696
MyoD	TransAM® MyoD	1 x 96-well plate	47196
		5 x 96-well plates	47696
NFAT	TransAM® NFATc1	1 x 96-well plate	40296
		5 x 96-well plates	40796
NFκB	TransAM® Flexi NFκB Family[5]	2 x 96-well plates	43298
	TransAM® Flexi NFκB p50	1 x 96-well plate	41098
	TransAM® Flexi NFκB p65	1 x 96-well plate	40098
	TransAM® NFκB Family[5]	2 x 96-well plates	43296
	TransAM® NFκB p50	1 x 96-well plate	41096
		5 x 96-well plates	41596
	TransAM® NFκB p50 Chemi	1 x 96-well plate	41097
		5 x 96-well plates	41597
	TransAM® NFκB p52	1 x 96-well plate	48196
		5 x 96-well plates	48696
	TransAM® NFκB p52 Chemi	1 x 96-well plate	48197
		5 x 96-well plates	48697
	TransAM® NFκB p65	1 x 96-well plate	40096
		5 x 96-well plates	40596
	TransAM® NFκB p65 Chemi	1 x 96-well plate	40097
		5 x 96-well plates	40597
NF-YA	TransAM® NF-YA	1 x 96-well plate	40396
		5 x 96-well plates	40896
Nrf2	TransAM® Nrf2	1 x 96-well plate	50296
		5 x 96-well plates	50796
Oct-4	TransAM® Oct-4	1 x 96-well plate	42496
		5 x 96-well plates	42996
p53	TransAM® p53	1 x 96-well plate	41196
		5 x 96-well plates	41696
PPARγ	TransAM® PPARγ	1 x 96-well plate	40196
		5 x 96-well plates	40696
Sp1 & Sp3	TransAM® Sp1	1 x 96-well plate	41296
		5 x 96-well plates	41796
	TransAM® Sp1/Sp3	1 x 96-well plate	40496
		5 x 96-well plates	40996
STAT	TransAM® STAT Family[6]	2 x 96-well plates	42296
	TransAM® STAT3	1 x 96-well plate	45196
		5 x 96-well plates	45696
T-bet	TransAM® T-bet	1 x 96-well plate	51396
		5 x 96-well plates	51896

1. AP-1 Family includes: c-Fos, FosB, Fra-1, c-Jun, JunB & JunD
2. GATA Family includes: GATA-1, GATA-2 & GATA-3
3. HNF Family includes: HNF-1, HNF-3α, HNF-3β & HNF-4α
4. MAPK Family includes: ATF-2, c-Jun, c-Myc, MEF2 & STAT1α
5. NFκB Family includes: p50, p52, p65, c-Rel & RelB
6. STAT Family includes: STAT1α, STAT3, STAT5A & STAT5B

For more information and a complete, up-to-date product listing, please call or visit us at www.activemotif.com/transam.