Simplified quantitation of activated transcription factors

TransAM™ Kits are highly sensitive enzyme-linked immunosorbent assay (ELISA)-based assays that facilitate the study of transcription-factor activation in mammalian tissue and cell extracts. They combine a fast, user-friendly format with a sensitive, specific assay. TransAM assays are up to 100 times more sensitive than gel-shift techniques and can be completed in less than five hours. The TransAM method eliminates the use of radioactivity and provides quantitative results. Moreover, the high-throughput stripwell format allows simultaneous screening of 1–96 samples.

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Until now, transcription factors have been studied using primarily three methods: gel-shift assays, western blotting and reporter-gene assays. These methods are time-consuming and provide, at best, only semiquantitative results. Moreover, they do not support high-throughput methods and lack sensitivity, specificity and reproducibility. The TransAM technology[1] eliminates these shortcomings, making transcription-factor research faster and more precise. Inconsistencies owing to variable reporter plasmid transfections are eliminated, along with the need to construct stable cell lines. The TransAM technology is available for the analysis of over 30 different transcription factors, including NFκB, AP-1, HIF and STAT.

A simple procedure

TransAM Kits offer a new method for rapid, sensitive and quantifiable measurement of transcription-factor activation. TransAM Kits are available in the original format, which comprises a 96-well plate on which oligonucleotides containing a consensus binding site have been immobilized, or in a new Flexi format, which enables a researcher to bind any oligonucleotide to the plate. In both formats, cell extract is added to each well, and the transcription factor of interest binds specifically to the oligonucleotide that is coated on the plate. Each well is then incubated with primary antibody specific for the active form of the bound transcription factor. Subsequent incubation with horseradish peroxidase (HRP)-conjugated secondary antibody and standard or chemiluminescent developing solution provides an easily quantified, sensitive readout (**Fig. 1**).

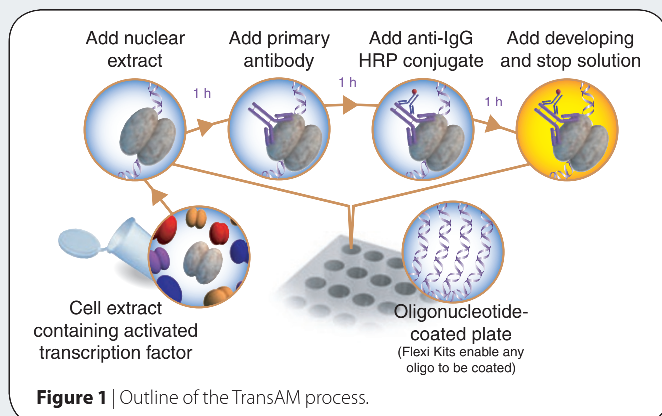

Figure 1 | Outline of the TransAM process.

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Activation and translocation of NFκB p50

We demonstrated the use of the simple TransAM DNA-binding ELISA to study the translocation and DNA-binding activity of the NFκB p50 transcription factor.

In the majority of cells, the five subunits of the NFκB family exist in an inactive form in the cytoplasm, bound to the inhibitory IκB proteins. Treatment of cells with various inducers results in the phosphorylation, ubiquitination and subsequent degradation of IκB proteins[2]. This leads ultimately to the translocation of NFκB dimers to the nucleus, where they activate appropriate target genes by binding to their target sequence. The original format of the TransAM NFκB p50 Kit was used to quantify the DNA-binding activity of NFκB p50 in nuclear and cytoplasmic extracts made from both unstimulated and TNF-α–stimulated HeLa cells. Because cytoplasmic NFκB p50 is inactive and cannot bind DNA, only the stimulated nuclear extract samples should bind to their target sequence. We also used competitive binding assays to demonstrate the assay's specificity for NFκB p50.

ELISA-based measurement of NFκB DNA-binding activity

We prepared nuclear and cytoplasmic extracts from unstimulated and TNF-α–stimulated HeLa cells using Active Motif's Nuclear Extract Kit. Then we added increasing amounts of these samples to the TransAM NFκB p50 Kit's 96-well assay plate. Each well in the

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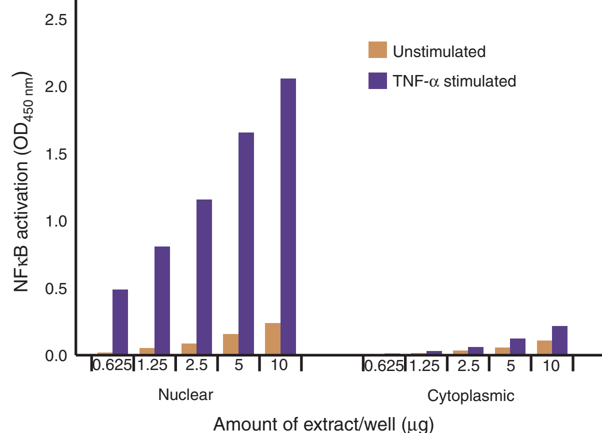

Figure 2 | DNA-binding activity of NFκB. Increasing amounts of nuclear and cytoplasmic extracts prepared from HeLa cell samples that were unstimulated or stimulated with 20 ng/ml TNF-α for 30 min were assayed for NFκB p50 DNA-binding activity using the TransAM NFκB p50 Kit. As activated NFκB translocates to the nucleus, only nuclear extract from stimulated samples should contain activated NFκB.

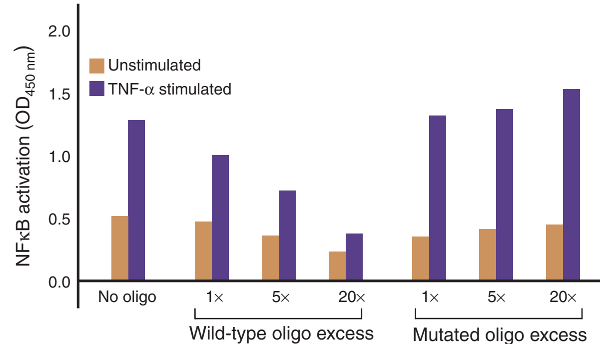

Figure 3 | Testing the specificity of the interaction. DNA-binding assays are performed in the presence of wild-type or mutated competitor oligonucleotides using 5 μg of nuclear extract from HeLa samples that were unstimulated or stimulated with 20 ng/ml of TNF-α for 30 min. Signal decrease only in the presence of the wild-type oligonucleotide confirms that the assay specifically measures binding of p50 to its target site.

original TransAM format is supplied coated with oligonucleotide that contains a consensus binding site for NFκB (5′-GGGACTTTCC-3′). Activated NFκB dimers bind this consensus site, and are quantified through the use of p50 and HRP-conjugated secondary antibodies, which are read on a standard plate reader. For competitive binding experiments, the assay is performed in the presence of wild-type or mutated competitor oligonucleotides. As the wild-type oligonucleotide contains a viable binding site, its presence in the buffer reduces binding to the oligonucleotide adhered to the plate. The mutated competitor oligonucleotide does not affect binding to the plate because it contains three mutated bases, which destroy the NFκB binding site.

The data in **Figure 2** demonstrate that the TransAM NFκB p50 assay can be used to measure the DNA-binding activity of NFκB p50. The linearity of the data shows that the signal is directly proportional to the quantity of transcription factor present and demonstrates the quantitative results provided by TransAM. The results also confirm that stimulation results in both translocation and activation of the DNA-binding activity of NFκB p50 because only nuclear extracts from stimulated cells yielded a positive signal.

The TransAM NFκB assay is specific

We confirmed the specificity of the NFκB-DNA interaction through use of the wild-type and mutated competitor oligonucleotides provided in the kit (**Fig. 3**). Increasing amounts of wild-type competitor oligonucleotide reduced the signal of the nuclear extract. In contrast, addition of even a large excess of mutated competitor oligonucleotide, which does not contain a consensus binding site for NFκB, does not affect the signal strength. As the p50 antibody in the assay has already been shown to be specific for the p50 transcription factor by western blot assay (data not shown), the results demonstrate that the signal being measured is that of the p50 transcription factor, which is bound to its consensus binding site.

Conclusion

Although the extreme complexity of transcriptional regulation dictates that multiple study systems and numerous assay methods be used in drug-discovery efforts, the TransAM ELISA-based method has a unique combination of features (high-throughput format, compatibility with both cell and tissue samples, high sensitivity, ability to quantify only activated transcription factors) that make it a powerful addition to existing methods for studying transcription factors. The TransAM technology supplied by Active Motif is now available for the study of over 30 different transcription factors. Detailed information including over 300 product citations and downloadable manuals is available at http://www.activemotif.com/transam.

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