NEW

Luminex® Transcription Factor Multiplex Assays

bead-based assay system for multiplexing analyte-specific DNA binding activity

Transcription factors bind to specific DNA sequences near genes and control their expression by regulating the recruitment of RNA pol II and other *trans*-acting factors, as well as modulating changes in histone modifications. Aberrant transcription factor activity is linked to various pathologies including autoimmune diseases and cancer. Traditional methods to assay transcription factor activity, such as Western blots, EMSA, reporter assays and ELISA, have their limitations, primarily the inability to quantify DNA binding events or to simultaneously compare activity among members of the same transcription factor family. To overcome these limitations, Active Motif has partnered with Luminex®, the industry leader for multiplexing, to develop Transcription Factor Multiplex Assays for studies of AP-1 and NFκB transcription factor families. The assays are designed for use with either the Luminex® 200™ or MAGPIX® instruments for multiplexing of analyte-specific DNA binding events. These highly sensitive assays require small sample input and can be adapted for high-throughput analysis, enabling researchers to collect more data at a faster rate using less sample than afforded by traditional methods.

How Does the Assay Work?

The Transcription Factor Multiplex assays work as pull-down reactions where lysates are combined with a biotinylated consensus binding sequence for the transcription factor family of interest. Antibodies specific for each transcription factor analyte are conjugated to fluorescent-labeled magnetic beads and used to capture the transcription factor-bound DNA. The fluorescent label of the bead is specific to each analyte, enabling multiple analytes to be analyzed simultaneously within the same sample for multiplexing. Streptavidin-phycoerythrin (SA-PE) is used to bind the biotinylated oligo and determine the magnitude of signal (Figure 1). The readout from the SA-PE is proportional to the amount of transcription factor binding.

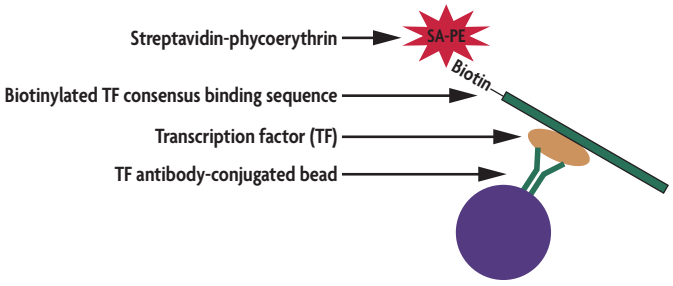


Figure 1: Bead-based Transcription Factor Multiplex assay. Illustration depicting the design of the Transcription Factor Multiplex assay as a bead-based pull-down assay for measuring levels of transcription factor-DNA binding activity.

To learn more about our Transcription Factor Multiplex Assays for use with Luminex instruments, please visit us at www.activemotif.com/luminex.

The Luminex® Principle

Each bead has a distinct fluorescent code that is associated with a unique bead number. Inside the Luminex 200 instrument, the beads are individually passed through a small shaft where a laser excites the internal fluorescent dye used for bead identification. A second laser is used to measure the magnitude of the SA-PE signal for quantification (Figure 2). The Luminex xPONENT software program provides a real-time readout of signal as median fluorescent intensity (MFI).

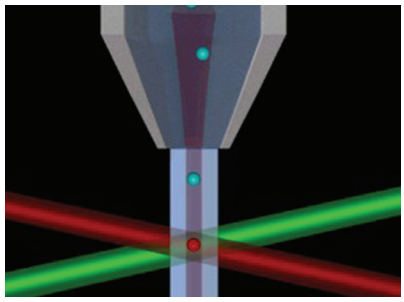


Figure 2: The Luminex 200™. The Luminex 200 instrument uses two distinct wavelengths to analyze individual beads as they pass through the machine for their bead identity and phycoerythrin signal.

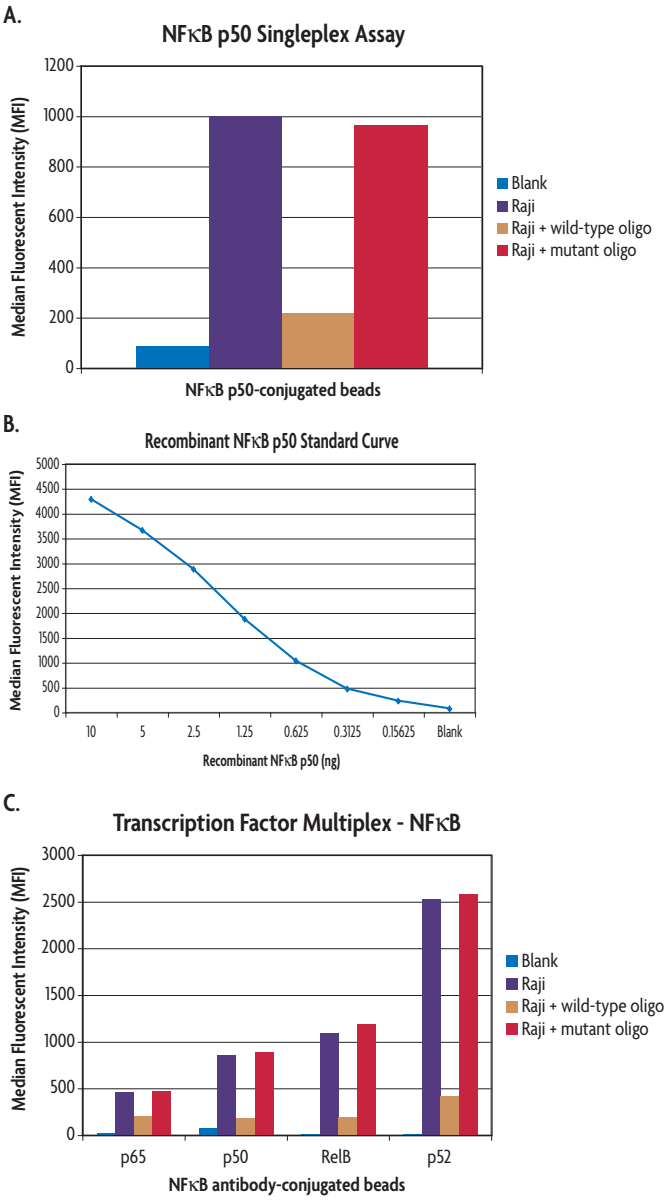
What's in the Box?

The **Transcription Factor Multiplex Kit** includes a 96-well assay plate, biotinylated oligonucleotide containing the DNA consensus binding sequence, assay buffers, control wild-type and mutant competitor oligos to confirm the specificity of the reactions, SA-PE and a positive control cell lysate.

The **Transcription Factor Antibody-conjugated bead(s)** are purchased separately to provide you the flexibility to customize analyte selection for your specific research needs.

Note: A complete assay requires the purchase of both the Transcription Factor Multiplex Kit and the antibody-conjugated bead(s) of interest.

Figure 3: Singleplex or multiplex analysis of NFκB DNA binding activity. The Transcription Factor Multiplex Kit – NFκB was used to assay DNA binding activity of NFκB analytes. Graph A shows singleplex data for NFκB p50. The positive control lysate, Raji nuclear extract, is tested in the absence or presence of the provided wild-type or mutant competitor oligos to confirm the specificity of the assay. Graph B shows a standard curve that was generated using recombinant p50 protein for normalization. Graph C shows a multiplex assay in which antibody-conjugated beads for multiple analytes were combined into a single well for simultaneous comparison of the activity of various NFκB proteins within the same sample. Comparison of the singleplex and multiplex graphs shows that multiplexing does not have any negative effects on the assay results.



Transcription Factor NFκB

NFκB transcription factors are widely studied due to their implication in the regulation of genes that control inflammation, cell proliferation and cell survival. The NFκB family is comprised of a mixture of homo- and heterodimeric subunits of the structurally related Rel family of transcription factors. NFκB p65 (RelA), RelB and c-Rel contain a transactivation domain that is required for translocation of active NFκB complexes into the nucleus where they bind to DNA. The p50 and p52 subunits do not contain the transactivation domain and must form heterodimers. Active Motif offers antibody-conjugated beads specific for NFκB p65, p50, RelB and p52 analytes (Figure 3).

Transcription Factor AP-1

AP-1 is composed of homodimeric and heterodimeric complexes of proteins of the Fos and Jun families that regulate the expression of genes involved in differentiation, proliferation and apoptosis. AP-1 expression is upregulated by various stimuli, including growth factors, phorbol esters and oncogenes. Phosphorylation by kinases leads to AP-1 transactivation and nuclear translocation where it binds to DNA and regulates transcription. Active Motif offers antibody-conjugated beads specific for c-Fos, FosB, JunB and JunD analytes.

TF Multiplex Advantages

- Multiplex analyte interrogation
- Customization of analyte selection
- Higher sensitivity than traditional methods
- High throughput processing
- Perform replicates to increase confidence in your results
- High specificity & lot-to-lot consistency
- Reduction in time, cost & labor
- Control oligos & lysates included to confirm assay specificity
- 3-hour assay time

Ordering Information

Product	Format	Catalog No.
Transcription Factor Multiplex Kit – AP-1	96 rxns	33100
c-Fos-conjugated beads	48 rxns	33101
FosB-conjugated beads	48 rxns	33102
JunB-conjugated beads	48 rxns	33104
JunD-conjugated beads	48 rxns	33105
Transcription Factor Multiplex Kit – NFκB	96 rxns	33110
RelB-conjugated beads	48 rxns	33111
NFκB p52-conjugated beads	48 rxns	33112
NFκB p65-conjugated beads	48 rxns	33113
NFκB p50-conjugated beads	48 rxns	33114

TO PLACE AN ORDER, call us or send an email to orders@activemotif.com.