Frequently Asked Questions

Transcription Factor Activation ELISA Kits [EKXXXX]

1. Can cell lysate be used with the TF ELISA Kits?

Unfortunately, we do not recommend the use of cell lysate with our TF ELISA Kits. Cell lysate will contain a large amount of cellular proteins. In addition, cell lysate will have a much lower proportion of active transcription factors than nuclear extract. For these reasons, we only recommend the use of nuclear extract with our kits.

2. Can the TF ELISA Kits be used with mouse or rat samples?

We have tested the PPARγ (tested with NIH3T3 cells, which is a mouse cell line) and MEF2 (tested with C2C12, which is a mouse cell line). There are a number of customers that have used the NFκBp50 Kit with mouse samples, and there are published references available for the use of the NFκBp50 kit with mouse samples. For cross-reactivity or reference information on our other TF ELISA kits, please contact USB Technical Support at 888-362-2447 or USBtechsupport@affymetrix.com.

3. Do the TF ELISA Kits contain standards?

Our kits are intended to measure DNA binding activity. Purified transcription factor (TF) proteins added to a well may not all bind to the DNA. Therefore, it is not possible to definitely determine how much signal is generated from a given amount of protein since unbound protein will be washed away during the wash steps. The TF ELISA Kits are used to determine fold change between different sample types.

4. How much protein should be loaded in each well?

The TF ELISA Kit requires a minimum of 5-10 µg loaded per well. We recommend that the protein concentration of sample inputs should be between 0.5 µg/µl to 2 µg/µl. An amount of 10 µl will be loaded per well.

5. What controls are included with the kits?

The TF ELISA Kits contain positive controls, which are most often nuclear extract from a cell line expressing the transcription factor of interest. A few of our TF ELISA Kits use recombinant transcription factor protein as the control. Our kits also contain a cold (competitive) control probe.

6. What is the cold (competitive) probe used for?

The cold probe is used to out-compete the labelled probe during a competition assay. The competition assay is used to verify the specificity of the probe, since the cold probe is the exact same sequence as the labelled probe.

7. Where can I find information on the antibodies used with the TF ELISA Kits?

The package insert for each TF ELISA Kit contains specific antibody information. You can find the package insert for each TF ELISA Kit in the Technical Documentation > Product Documentation section of www.affymetrix.com/support/technical/usb/index.affx.