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**User's Manual**

# FOXO1 (Phospho-Ser256) ELISA Kit

REF **DEIA-XYA689**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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# PRODUCT INFORMATION

## Principles of Testing

The DNA-Binding ELISA Kit contains components necessary for detection of active transcription factors in eukaryotic nuclear or cell lysates. This particular immunoassay utilizes the qualitative technique of an indirect ELISA. Streptavidin is bound to the immunoassay plate and specific biotinylated double- stranded (dsDNA) oligonucleotides are then added to bind to the streptavidin via a high affinity biotin-streptavidin interaction. After subsequent blocking of extraneous binding sites in each well, the sample containing the target of interest can be added. Primary antibody is added to bind activated transcription factors bound to the dsDNA oligonucleotide, which has been immobilized via the plate- coated streptavidin. A HRP-conjugated secondary antibody specific for rabbit IgGs is added, which allows for specific binding to the Primary Antibody, and consequently colorimetric detection upon addition of the TMB substrate.

For color development, TMB (3, 3', 5, 5'-Tetramethylbenzidine) is added to each well. After addition of the substrate, a peroxidase catalyzed reaction will produce a blue TMB Diimine product that is proportional to the target concentration in the sample. Color development is quenched by addition of Stop Solution, or 2 N Sulfuric Acid, which turns the solution yellow. The absorbance can then be read by a spectrophotometer at 450 nm and subsequently allowing for determination of the target concentration in the sample.

Currently, the most common methods to detect transcription factor binding to DNA elements and motifs are electrophoretic mobility shift assays (EMSAs), chromatin immunoprecipitation, western blotting, and expression of fused target and reporter genes. These methods are often time consuming, complicated, and make it difficult to achieve satisfactory results. Assay Biotech TFactTM DNA-Binding ELISA Kits can significantly reduce the necessary runtime to within one day and eliminate the need for harmful radioactive labeling while maintaining high sensitivity and signal-to-noise ratio. In the past, it was strenuous and inefficient to perform high-throughput screening for hundreds of different samples or transcription factors. Today, our revolutionary TFactTM DNA-Binding ELISA Kits can eliminate these challenges and help expedite the journey from research to publication or product.

## Materials Required But Not Supplied

□ Microplate reader able to measure absorbance at 450 nm (with correction wavelength set to 540 nm or 570 nm)

□ Micropipettes capable of measuring volumes from 1 µl to 1 ml

□ Deionized or sterile water (ddH2O)

□ Sterile 1x PBS and 5 M NaCl for nuclear lysate preparation

□ Squirt bottle, manifold dispenser, multichannel pipette reservoir, or automated microplate washer

□ Graph paper or computer software capable of generating or displaying logarithmic functions

□ Absorbent paper or vacuum aspirator

□ Test tubes or microfuge tubes capable of storing ≥1 ml

□ Bench-top centrifuge (optional)

□ Bench-top vortex (optional)

□ Orbital shaker (optional)

## Storage

4° C/6 Months.

After receiving the kit, please open and store the kit components at the temperature indicated in **Reagents And Materials Provided**. If used frequently, reagents may be stored at 4°C. Reconstituted Nuclear Lysate Positive Control must be aliquoted and stored at -80□C.

## Specimen Collection And Preparation

The DNA-Binding ELISA Kit allows for the detection and qualitative analysis of endogenous levels of activated transcription factors in a variety of nuclear and cell lysates. All preparations of experimental samples should maintain the natural and active form of the target transcription factor. In this kit, all necessary buffers and reagents are provided for nuclear extraction from cell culture.

Tissue homogenates and heterogeneous mixtures may contain contaminants which interfere with the assay, hence it is best to test for interference by using at least two different dilutions of the sample. If testing demonstrates good correlation between concentration/dilution factor and OD reading, purification may not be required. However, if good correlation is not achieved or seen, further purification is advised. Moreover, if samples contain any visible precipitate, they must be centrifuged for 10 minutes at ≥10,000 x g prior to use in the assay.

It is always recommended to make several dilutions to obtain the best OD reading. Ideal OD readings will fall within the detectable range of the assay, which is dependent on the spectrophotometer used. It is up to the investigator to determine an appropriate dilution factor and recommended to run each dilution in duplicates. A minimum of 100 µl of sample or diluted sample is required for each well; please adjust dilution volumes accordingly.

If samples are ready to be used within 24 hours, aliquot and store at 4°C. If samples are to be saved for future or long term use, aliquot into multiple tubes and store at -80°C. Avoid repeated freeze/thaw cycles to prevent loss of biological activity of transcription factors in experimental samples.

If a sample contains any visible precipitate or pellet, it must be clarified prior to use in the assay.

## Reagent Preparation

The following reagents will need to be prepared prior to start of the assay:

**1x Wash Buffer**

The Wash Buffer is provided at 10x concentration. To prepare 1x Wash Buffer, add 50 ml of 10x Wash Buffer into 450 ml of ddH2O for a final volume of 500 ml of 1x Wash Buffer.

**Nuclear Lysate Positive Control**

The Binding Buffer is provided at 2x concentration. It is recommended to make fresh 1x Binding Buffer for the reconstitution of Nuclear Lysate Positive Control. Add 60 µl of 2x Binding Buffer to 60 µl ddH2O to make 120 µl of 1x Binding Buffer. Add 100 µl of 1x Binding Buffer into the Nuclear Lysate Positive Control tube. The Nuclear Lysate Positive Control should be kept on ice at all times. Aliquot and store at -80□C (long term storage) and avoid freeze/thaw cycles if not immediately used.

**1x Primary Antibody and 1x Primary Phospho-Antibody**

The Primary Antibody and Primary Phospho-Antibody are provided at 100x concentration. It is recommended to make a fresh 1x Antibody solutions. Add 100 µl of 100x Primary Antibody or Primary Phospho-Antibody into 9.9 ml of Primary Antibody Diluent to make enough 1x Primary Antibody or 1x Primary Phospho-Antibody solution for one 96-well microplate.

**Aliquoting of Buffers and Reagents**

If you do not plan on using the whole kit in one sitting, it is recommended to aliquot the buffers and reagents, reconstituted Nuclear Lysate Positive Control, 2x Binding Buffer, 100x Protease and Phosphatase Inhibitors, Cytoplasmic Extraction Buffer, Nuclear Wash Buffer, Nuclear Extraction Buffer, etc. and store them at the temperatures indicated in Reagents And Materials Provided.

HRP-Conjugated Anti-Rabbit IgG Secondary Antibody, Ready-to-Use Substrate, Stop Solution, Primary Antibody Diluent, Wild-Type (WT) Consensus dsDNA Oligonucleotide, Mutant (MT) Consensus dsDNA Oligonucleotide, Nuclear Wash Buffer, Cytoplasmic Extraction Buffer, Nuclear Extraction Buffer are ready-to-use.

## Precautions

_ This kit and its components should be handled by those trained in laboratory techniques and used according to the principles of good laboratory practice.

_ Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin. Please carefully review the MSDS for each reagent before conducting the experiment.

## Limitations

 This ELISA kit is intended for research purposes only, NOT diagnostic or clinical procedures of any kind.

 Materials included in this kit should NOT be used past the expiration date on the kit label.

 Reagents or substrates included in this kit should NOT be mixed or substituted with reagents or substrates from any other kits.

 Variations in pipetting technique, washing technique, operator laboratory technique, kit age, incubation time or temperature may cause differences in binding affinity of the materials provided.

 The assay is designed to eliminate interference and background by other cellular macromolecules or factors present within any biological samples. However, the possibility of background noise cannot be fully excluded until all factors have been tested using the assay kit.