

## c-Jun Phospho (pT91/93) Sandwich ELISA Kit USER MANUAL

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## 2.0 INTRODUCTION

c-Jun is a transcription factor that recognizes and binds to the enhancer heptamer motif 5'-TGA [CG] TCA-3'. c-Jun forms homodimers and heterodimers with Fos and other jun-related proteins which, together, comprise the AP-1 transcription factor that binds TPA response elements (TREs). c-Jun therefore mediates transcriptional regulation in response to a variety of stimulants (1). c-Jun is tightly regulated posttranslationally and is phosphorylated in two distinct regions. Ser 63 and 73 are required to be phosphorylated for efficient transactivation function. The kinases responsible for this modification in vivo are the SAPK/JNKs (2, 3).

Transactivation of c-Jun is regulated by Jun-N-terminal kinases (JNKs) through phosphorylation at serine 63 and 73 (S63/S73), as well as at threonine 91 and 93 (T91/T93). The integrity of JNK phosphorylation sites at serines 63/73 and at threonines 91/93 in c-Jun is essential for signal-dependent target gene activation (4). These two groups of phosphoacceptor sites respond to different grades of genotoxic stress. c-Jun phosphorylation is restricted to S63/S73, following a short exposure to the DNA-damaging compound etoposide. In contrast, JNK-dependent phosphorylation of T91/T93 requires continuous exposure to the drug (5).

## Principle of the c-Jun Phospho (pT91/93) Sandwich ELISA

This assay employs the sandwich enzyme immunoassay technique that detects endogenous level of c-Jun when phosphorylated at Threonine 93. Rabbit anti-c-Jun monoclonal antibody has been pre-coated onto the microplate. Cell lysate is pipetted into the wells. Following extensive washing, biotinylated rabbit anti-c-Jun Phospho (pT93) monoclonal antibody reagent is added. Following a wash to remove any unbound antibody reagent, streptavidin-HRP is added to the well. After washing away the unbound streptavidin-HRP, a substrate solution is added to the wells to develop color. The magnitude of the absorbance for this developed color is proportional to the amount of endogenous phosphorylated c-Jun.

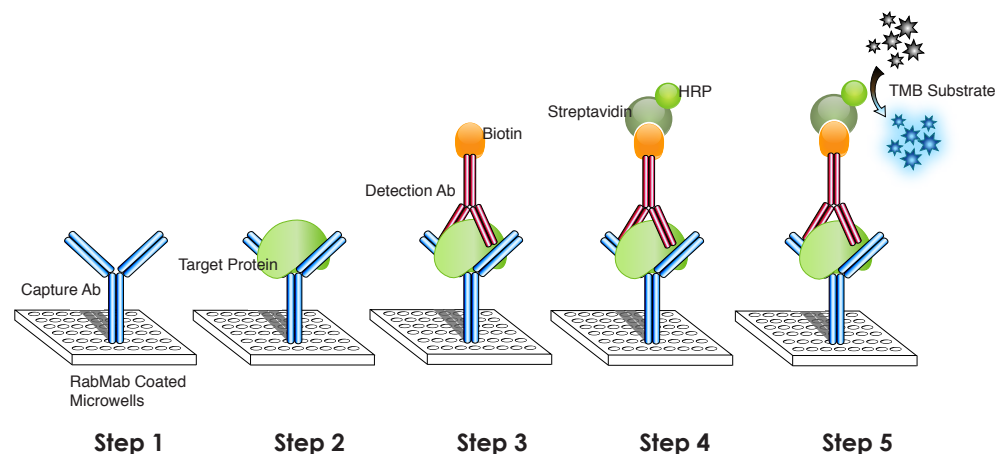


Fig 1. 5 Steps of Sandwich ELISA assay.

### 3.0 MATERIALS, REAGENTS AND EQUIPMENT

**Table 1 – Components/Reagents Provided**

Reagent	Quantity	Storage	Color
Anti-c-Jun Ab Coated Microwells	96 wells	4 - 8° C	
Biotinylated Anti-c-Jun Phospho (pT91/93) Antibody Reagent (1x)	11 ml	4 - 8° C	
Streptavidin-HRP Reagent (100x)	120 ul	4 - 8° C	
Antigen/Antibody Diluent Buffer (1x)	20 ml	4 - 8° C	white
ELISA Washing Buffer (10x)	25 ml	4 - 8° C	blue
TMB A Substrate Solution (1x)	7 ml	4 - 8° C	brown
TMB B Substrate Solution (1x)	7 ml	4 - 8° C	brown
Stop Solution (1x)	11 ml	4 - 8° C	red

#### Required Components/Reagents Not Provided

- Deionized water

#### Required Equipment Not Provided

- Pipettors and pipet tips of various sizes
- Vortex mixer or equivalent
- Rotating shaker
- Microtiter plate reader

### 3.1 NOTES ON MATERIALS

#### Microtiter Plate

- Bring stripped microtiter plate to room temperature. Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate by evenly pushing the bottoms of the microwell strips.
- Store extra strips immediately in the sealed bag at 4°C.













#### Buffers




- Bring ELISA washing buffer (10x) to room temperature before diluting with Mili-Q or equivalent deionized water.
- Store all buffer and reagents at 4°C when not in use.
- Dilute Streptavidin-HRP Reagent (100x) with Antigen/Antibody Diluent Buffer (1x).

### 4.0 PROCEDURE

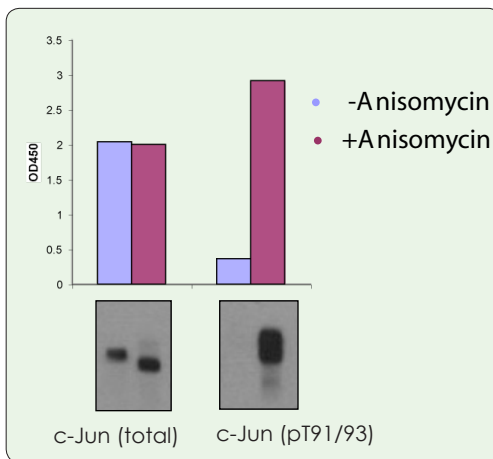
(Please read through entire procedure before beginning)

#### 4.1 ELISA Protocol

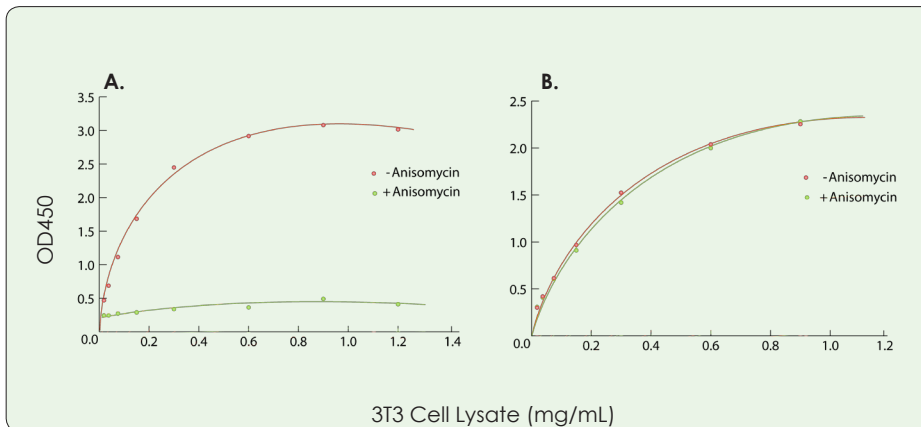
- Prepare all reagents and samples as directed in the previous section and bring to room temp.
- Remove excess microplate strips from plate and return to foil pouch.
-   Add 100 ul of each diluted cell lysate to the appropriate well. Incubate for 1 hr. at room temp. (18-25°C) on a shaker.
-  Aspirate each well and wash 3 times with 200 ul 1x wash buffer per well.
-   Add 100 ul of Biotinylated Anti-c-Jun Phospho (pT91/93) Antibody Reagent (1x) to each well. Incubate for 1 hr. at room temp. on a shaker.
-  Aspirate each well and wash with 200 ul 1x Wash Buffer per well. Repeat wash 2 additional times.
-   Add 100 ul of Streptavidin-HRP Reagent (1x) to each well. Incubate for 30 min. at room temp. on a shaker.
-  Aspirate each well and wash with 200 ul 1x Wash Buffer per well. Repeat wash 2 additional times.
-   i. Combine TMB A and TMB B (1:1)\*  
\* Volume of each TMB substrate needed = 50 ul (# of wells +1)  
Add 100 ul of combined substrate solution to each well. Incubate for 30 min. (max) at room temp. on a shaker.
-  j. Add 100 ul of Stop Solution to each well.
- k. Determine the optical density at 450 nm using a microplate reader within 30 minutes.

 add     incubate     wash

## 5.0 Typical Data



**Fig 2.** Treatment of 3T3 cells with Anisomycin stimulates phosphorylation of c-Jun at Threonine 93, detected by sandwich ELISA kits of c-Jun (total) and Phospho c-Jun pT91/93 and western blot.



**Fig 3A.** Relationship between protein concentration of lysates from Anisomycin-treated 3T3 cells and phospho c-Jun (T93) kit assay optical density readings.

**Fig 3B.** Relationship between protein concentration of lysates from Anisomycin-treated 3T3 cells and c-Jun (total) kit assay optical density readings.

## 6.0 SENSITIVITY / SPECIFICITY

c-Jun Phospho (pT91/93) Sandwich ELISA Kit detects endogenous levels of c-Jun phosphorylated at Thr 91/93. As shown in Figure 2 and Figure 3, a significant induction of c-Jun Phospho (pT91/93) in 3T3 cells treated with Anisomycin is detected. However, the level of total c-Jun (phospho and non-phospho), detected by western blot, remains unchanged.

## 7.0 REFERENCES

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4. Weiss C, Schneider S, Wagner EF, Zhang X, Seto E, Bohmann D. (2003) EMBO J. 22(14):3686-95
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