

## ZAP-70 Total Sandwich ELISA Kit USER MANUAL

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### Principle of the ZAP-70 Total Sandwich ELISA

This assay employs the sandwich enzyme immunoassay technique that detects endogenous levels of ZAP-70. Rabbit anti-ZAP-70 monoclonal antibody has been pre-coated onto a microplate. Cell lysates are pipetted into the wells. Following extensive washing, biotinylated rabbit anti-ZAP-70 monoclonal antibody reagent is added to detect ZAP-70. Following a wash to remove any unbound antibody reagent, streptavidin-HRP is added to the well. After washing away the unbound streptavidin-HRP, TMB substrate is added to the wells to develop color. The magnitude of the absorbance for this developed color is proportional to the quantity of ZAP-70 protein.

### 2.0 INTRODUCTION

ZAP-70, a Syk-family protein tyrosine kinase, plays a critical role in mediating T cell signal transduction in response to T cell receptor (TCR) activation (1). TCR-mediated activation of the Src-family kinases, Lck and Fyn, results in tyrosine phosphorylation of the TCR zeta and CD3 chains. These domains serve as targets for binding of ZAP-70 via its tandem SH2 domains. This binding correlates with activation of ZAP-70, a critical event in T cell activation (2). Following TCR engagement, ZAP-70 is phosphorylated on several tyrosine residues, presumably by two mechanisms: an autophosphorylation and a trans-phosphorylation by the Src-family tyrosine kinase, Lck. Lck phosphorylates Tyrosine 492 and 493 located at the activation loop of the catalytic domain of ZAP-70, leading to its activation and increased autophosphorylation (3).

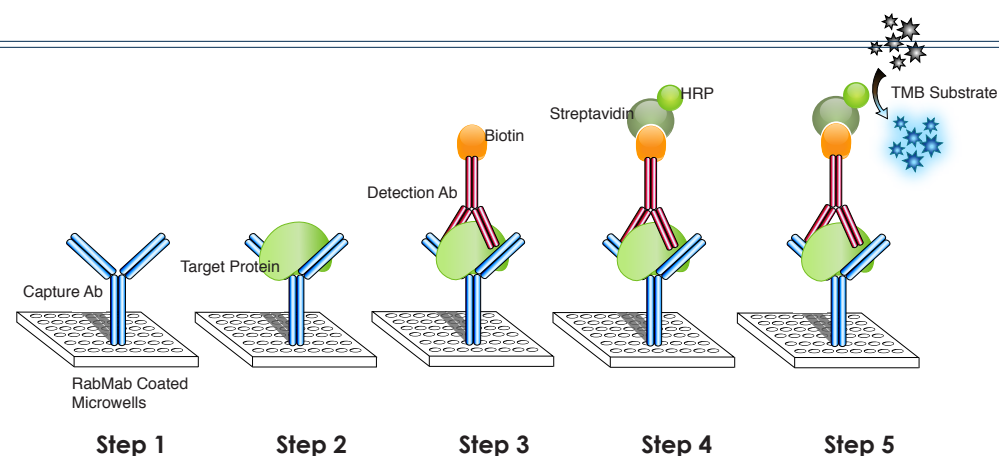


Fig 1. 5 Steps of Sandwich ELISA assay.

### 3.0 MATERIALS, REAGENTS AND EQUIPMENT

**Table 1 – Components/Reagents Provided**

Reagent	Quantity	Storage	Color
Anti-ZAP-70 Ab Coated Microwells	96 wells	4 - 8° C	
Biotinylated Anti-ZAP-70 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-ZAP-70 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.
-  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.
-  Add 100 ul of Stop Solution to each well.
- Determine the optical density at 450 nm using a microplate reader within 30 minutes.



add

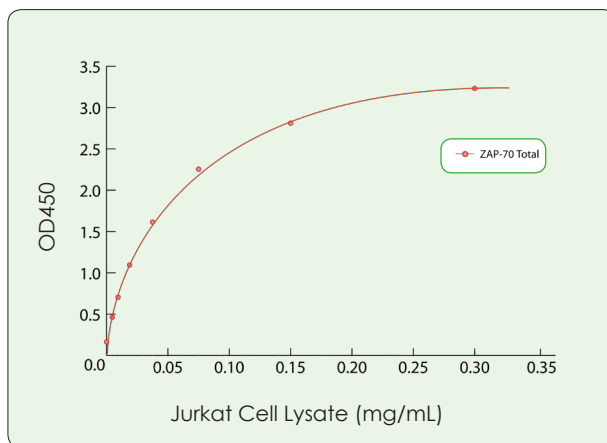


incubate

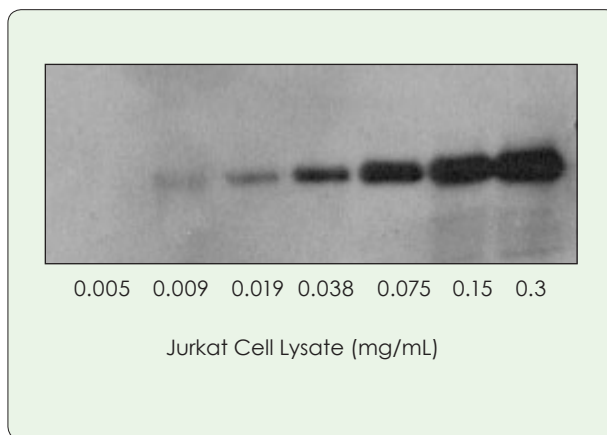


wash

## 5.0 Typical Data



**Fig 2.** The relationship between protein concentration of lysates from Jurkat cells and the absorbance at 450 nm is shown



**Fig 3.** Western Blot image of ZAP-70 protein in Jurkat lysate used for ELISA.

## 6.0 SENSITIVITY / SPECIFICITY

Total ZAP-70 sandwich ELISA kit detects endogenous levels of ZAP-70 total protein.

## 7.0 REFERENCES

1. Chu, D.H., et al. The Syk family of protein tyrosine kinases in T-cell activation and development. *Immunol. Rev.* 165: 167
2. Isakov, N., et al. ZAP-70 binding specificity to T cell receptor tyrosine-based activation motifs: the tandem SH2 domains of ZAP-70 bind distinct tyrosine-based activation motifs with varying affinity. *J Exp Med.* 181: 375
3. Di Bartolo, V., et al. Tyrosine 319, a newly identified phosphorylation site of ZAP-70, plays a critical role in T cell antigen receptor signaling. *J Biol Chem.* 274: 6285

## 8.0 CONTACT

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