

PBK Sandwich ELISA Kit USER MANUAL

1.0 TABLE OF CONTENTS

2.0 Introduction	p.1
3.0 Materials & Reagents	p.3
3.1 Notes on Materials	p.3
4.0 Protocols	p.4
4.1 ELISA Procedure	p.4
5.0 Typical Data	p.5
6.0 Sensitivity / Specificity	p.5
7.0 References	p.6
8.0 Contact Information	p.6
9.0 Disclaimer / Warranty	p.6

2.0 INTRODUCTION

PDZ-binding kinase (PBK) or TOPK, an intermediate in the Ras-MAPK Kinase signaling, is upregulated in a variety of neoplasms including hematological malignancies. Studies provide a plausible explanation for the role of PBK augmenting tumor cell growth following transient appearance in different types of progenitor cells in vivo (1). PBK is a newly identified member of a novel MEK3/6-related MAPKK that may be enrolled in the activation of lymphoid cells and support testicular functions (2). In vitro, PBK binds specifically to PDZ2 of hDlg through its C-terminal T/SXV motif. PBK and hDlg are phosphorylated at mitosis in HeLa cells, and the mitotic phosphorylation of PBK is required for its kinase activity. In vitro, cdc2/cyclin B phosphorylates PBK. This evidence shows how PBK could link hDlg or other PDZ-containing proteins to signal transduction pathways regulating the cell cycle or cellular proliferation (3).

Principle of the PBK Sandwich ELISA

This assay employs the sandwich enzyme immunoassay technique that detects endogenous levels of PBK (**Fig 1**). Rabbit anti-PBK monoclonal antibody has been pre-coated onto a microplate (**Step 1**). Cell lysate is pipetted into the wells (**Step 2**). Following extensive washing, biotinylated rabbit anti-PBK monoclonal antibody reagent is added (**Step 3**). Following a wash to remove any unbound antibody reagent, streptavidin-HRP is added to the well (**Step 4**). After washing away the unbound streptavidin-HRP, a substrate solution is added to the wells to develop color (**Step 5**). The magnitude of the absorbance for this developed color is proportional to the amount of endogenous PBK protein.

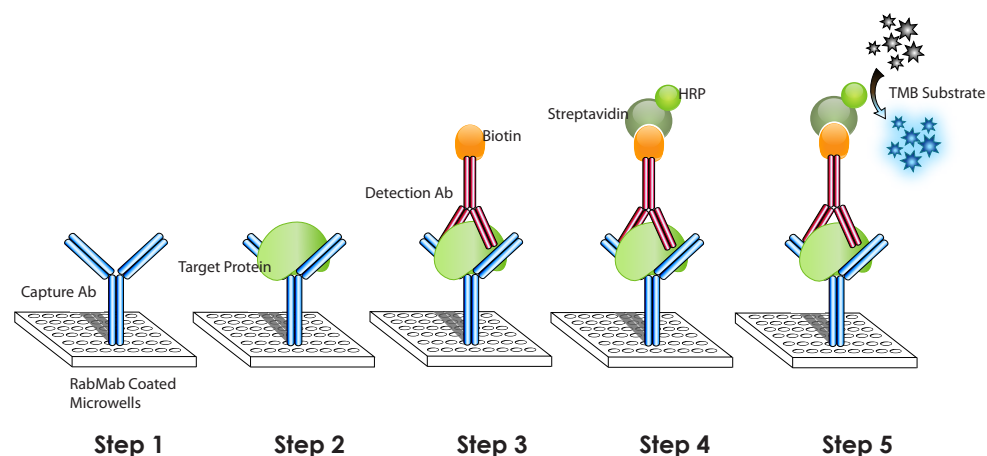


Fig 1. 5 Steps of Sandwich ELISA assay.

3.0 MATERIALS, REAGENTS AND EQUIPMENT

Table 1 – Components/Reagents Provided

Reagent	Quantity	Storage	Color
Anti-PBK Ab Coated Microwells	96 wells	4 - 8° C	
Biotinylated Anti-PBK 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 PBK Antibody Reagent (1x) to each well. Incubate for 1 hr. at room temp. (18-25°C) 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 to each well. Incubate for 30 min. at room temp. (18-25°C) 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. (18-25°C) 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

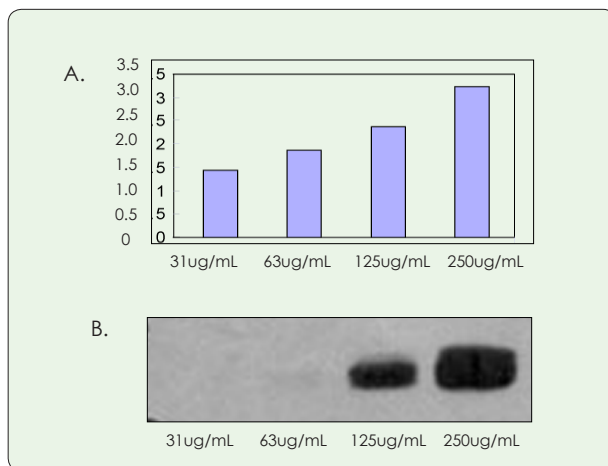


Fig 2a. Relationship between protein concentration of HeLa cell lysates and kit assay optical density readings.

Fig 2b. The amount of protein used for ELISA is shown in Western Blot.

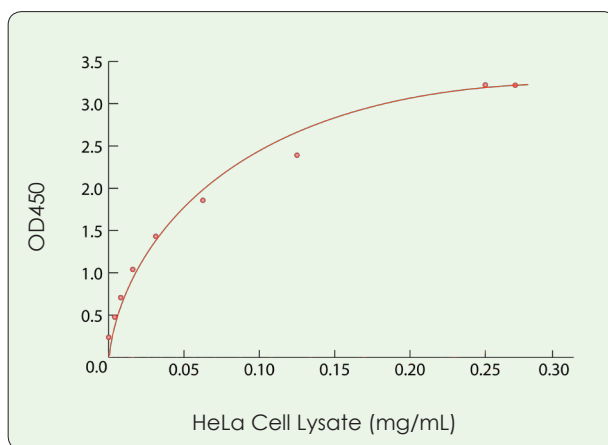


Fig 3. The relationship between the protein concentration of lysates from HeLa cells and assay optical density readings.

6.0 SENSITIVITY / SPECIFICITY

PBK Sandwich ELISA Kit detects a level of PBK in cell lysates (Fig. 2 & 3).

7.0 REFERENCES

1. Nandi AK, et al. Biochem Biophys Res Commun 358(1): 181-1 (2007)
2. Abe Y, et al. J Biol Chem 275(28):21525-31 (2000)
3. Gaudet S, et al. Proc Natl Acad Sci 97(10):5167-72 (2000)

8.0 CONTACT

Epitomics, Inc.
 863 Mitten Road Suite 103
 Burlingame, CA 94010-1303, U.S.A.
 Phone: (877) 772-2622 (Toll Free North America);
 (650) 583-6688
 Fax: (650) 583-6680

www.epitomics.com

9.0 DISCLAIMER* / WARRANTY

Epitomics, Inc. products are warranted to meet the applicable product specifications described. Notice of non-conforming products should be made to Epitomics, Inc. within 21 days of receipt of the product. Epitomics, Inc. disclaims any and all responsibility for any injury or damage which may be caused by the fault of the user or the buyer in accordance with the terms and conditions herein.

* Important Note:

This product as supplied is intended for research use only. Not for therapeutic or diagnostic applications.