

## Human Menin ELISA Kit

For the quantitation of human Menin concentrations in cells, PBMC and cell lysates.

### INTRODUCTION

Menin, also known as MEN1, is an essential component of a MLL/SET1 histone methyltransferase (HMT) complex that specifically methylates 'Lys-4' of histone H3 (H3K4). Menin is a transcriptional regulator. It is involved in TGFB1-mediated inhibition of cell-proliferation, possibly regulating SMAD3 transcriptional activity.

Human Menin ELISA is a solid phase ELISA designed to measure human Menin levels in cell culture supernatants, serum, plasma, and cell lysates. The main feature is that **the kit makes the assay simple, easy, accurate, and fast. The measurement can be finished in 3 hours, not 5-6 hours (Fig. 1), and only includes one washing step before color development.** The detection range is from 1.638 to 400 ng/mL. The levels of human Menin samples are parallel to the standard curves obtained using the kit standards linearly. These results indicate that this kit can be used to determine relative mass values for human Menin protein.

### PRINCIPLE OF THE ASSAY

This assay employs our novel proprietary sandwich enzyme immunoassay techniques (See Fig. 1). A monoclonal antibody specific for human Menin was pre-coated onto a microplate. Standards or samples are pipetted into the wells. After incubation, Biotinylated detection antibody is added to the well to form a sandwich complex. Simply aspirate each well without wash, directly add Streptavidin-HRP into the complex. Following a wash, an ultra-sensitive TMB substrate solution is added to the wells for color develops. The color intensity is in proportion to the amount of Menin bound in the initial step. The intensity of the color is measured by plate read at 450 nm.

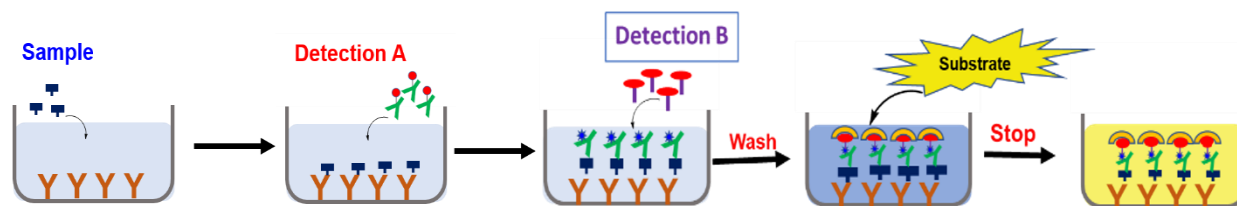


Fig.1 Simple ELISA procedure.

### KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Human Menin Microplate	TBS32100A	96 well microplate (12 strips of 8 wells) coated with a Capture Antibody specific for human Menin.	The unused wells can be stored in the sealed foil pouch containing the desiccant pack for up to 1 month at 2-8 °C.
Human Menin Standard	TBS32100B	60 µL of Recombinant human Menin protein (20ug/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS32100C	12 mL of human biotinylated Menin antibody.	May be stored for up to 3 months at 2-8 °C.
Detection B	TBS32100D	12 mL of Streptavidin-HRP	
Assay Diluent	TBS32100E	12 mL of a buffered protein base with preservatives.	
10x Wash Buffer	TBS3000W	12 mL of concentrated solution (10x).	
TMB Substrate	TBS3000T	12 mL of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 mL of 2 N sulfuric acid.	

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.  
The kit contains sufficient materials to run an ELISA on one 96 well plate.

### PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

**REAGENT PREPARATION**

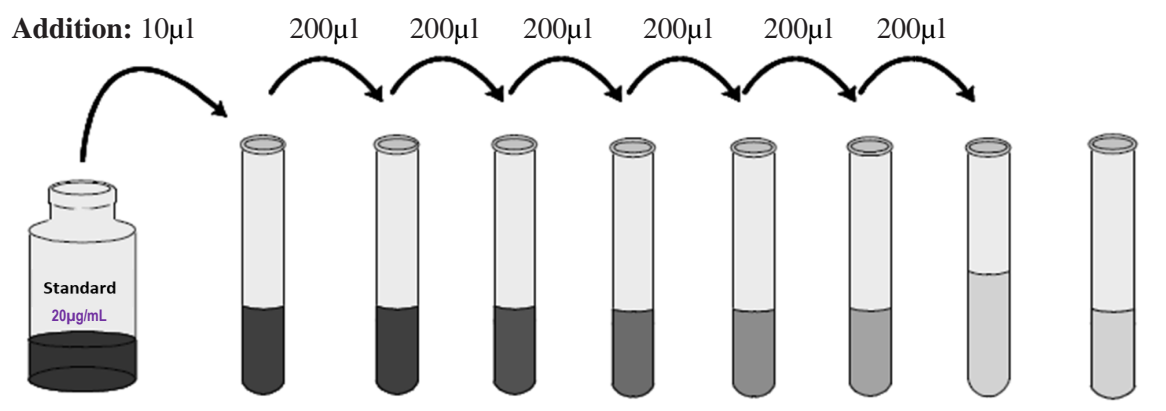
Bring all reagents to room temperature before use.

**Wash Buffer:** Add 10 mL of Wash Buffer Concentrate (10x) to 90 mL of deionized distilled water to prepare 100 mL of Wash Buffer (If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.).

**Human Menin Standard Preparation:**

1. Label test tubes as #1 through #8. Pipet 490  $\mu\text{L}$  of 1x Assay Diluent into tube #1, and 300  $\mu\text{L}$  into tubes #2 to #8 as diagram below.
2. Add 10  $\mu\text{L}$  of the Human Menin Standard stock solution (20 $\mu\text{g}/\text{mL}$ ) by dilution of 50X to tube #1 and mix.
3. Make 2.5x serial dilutions of the standard using the 400ng/mL standard solution from tube #2 through #7 with sequential transfer of 200  $\mu\text{L}$  to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 400, 160, 64, 25.6, 10.24, 4.096 and 1.648 ng/mL. Tube# 8 is Standard 0.

**Fig.2 Diagram for Human Menin standard preparation**



Standard	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer ( $\mu\text{L}$ )	490	300	300	300	300	300	300	300
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol ( $\mu\text{L}$ )	10	200	200	200	200	200	200	0
Final Conc (ng/ml)	400	160	64	25.6	10.24	4.096	1.638	0

**ASSAY PROCEDURE**

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Add 80  $\mu\text{L}$  of standard, sample, or control per well, cover with the adhesive sealer. Incubate at **37°C for 1.5 hours**.
2. Aspirate each well (*no wash*). Invert the plate and blot it against clean paper towels.
3. Add 100  $\mu\text{L}$  of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **37°C for 60 min**.
4. Aspirate each well (*no wash*). Invert the plate and blot it against clean paper towels.
5. Add 100  $\mu\text{L}$  of **Detection B** to each well. Incubate at **37°C for 30 min**.
6. Aspirate each well, and wash for 3 times by filling each well with 300  $\mu\text{L}$  Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
7. Add 100  $\mu\text{L}$  of **TMB Substrate** to each well. Incubate **at RT for 10-20min** (*Protect from light*). The color turns blue. If the color is light, the incubation time can be longer.

8. Add 50µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
9. Determine the optical density of each well within 5 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

**CALCULATION OF RESULTS**

Average the duplicate readings for each standard, control, and sample subtract the average zero standard optical density (O.D.).

Create a standard curve using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human Menin concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

**TYPICAL DATA**

This standard curve ( $R^2=1.000$ ) is provided in Fig. 3 for demonstration only. A standard curve should be generated for each set of samples assayed.

**SENSITIVITY**

The minimum detectable dose (MOD) of human Menin is typically 0.6 ng/ml.

The Intra-CV is < 10%, the Inter-CV is < 10%.

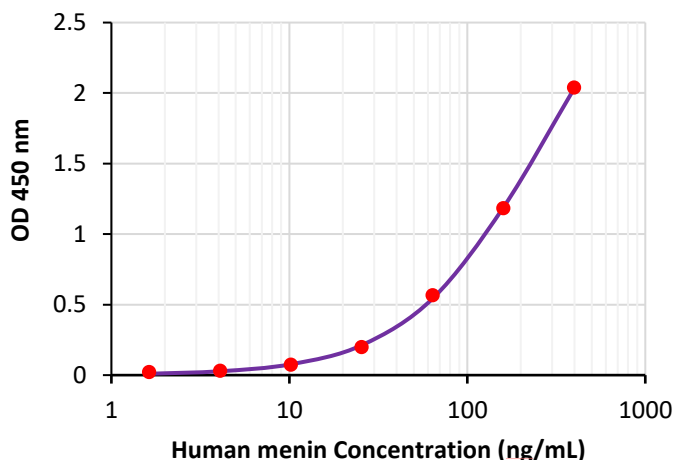
**SPECIFICITY**

This assay recognizes natural and recombinant human Menin.

**RELATIVE PRODUCTS**

- Human IL-1β ELISA (TBS3219)
- Human IL-2 ELISA (TBS3220)
- Human IL-4 ELISA (TBS3221)
- Human IL-6 ELISA (TBS3223)
- Human IL-7 ELISA (TBS3224)
- Human IL-8 ELISA (TBS3225)
- Human IL-13 ELISA (TBS3227)
- Human IL-17 ELISA (TBS3228)
- Human IL-22 ELISA (TBS3229)
- Human IFN-gamma ELISA (TBS3230)
- Human TGF-β1 ELISA (TBS3232)
- Human GM-CSF ELISA (TBS3233)
- Human MIP-1α ELISA (TBS3234)
- Human TNF-α ELISA (TBS3235)
- Human IL-18 ELISA (TBS3239)

**Fig. 3 Human menin Standard Curve**



**For research use only. Not for use in diagnostic procedures.**