Human Menin ELISA Catalog Number: TBS32100

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

INTRODUCTION

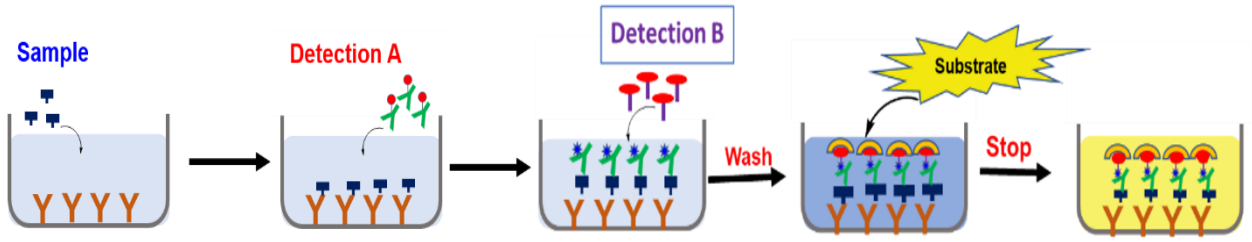
Menin, also known as MEN1, is an essential component of an MLL/SET1 histone methyltransferase (HMT) complex that specifically methylates 'Lys-4' of histone H3 (H3K4). Menin is a transcriptional regulator. It is involved in TGFB1-mediated inhibition of cell-proliferation, possibly regulating SMAD3 transcriptional 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 detection range is from 0.18 to 150 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 to human menin was pre-coated onto a microplate. Standards or samples are pipetted into the wells. After incubation, biotinylated menin detection antibody is added to the well to form a sandwich complex. After simply aspirating each, and washing, add Streptavidin-HRP into the complex. Following a wash, an ultra-sensitive TMB substrate solution is added to the wells for color development. 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	15 µL of Recombinant human Menin protein (10 µg/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	20 µL of human biotinylated Menin antibody (600x).	May be stored for up to 3 months at 2-8 °C.
Detection B	TBS32100D	40 µL of Streptavidin-HRP (300x)	
Assay Diluent	TBS32100E	25 mL of a buffered protein base with preservatives.	
10x Wash Buffer	TBS3000W	50 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

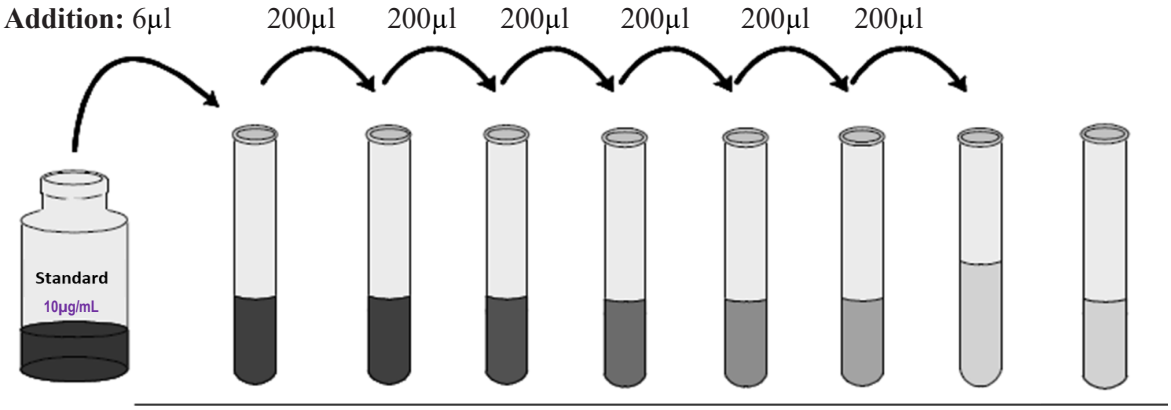
Bring all reagents to room temperature before use.

1. **Wash Buffer (1x):** Add 10 mL of 10x Wash Buffer Concentrate 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*).
2. **Detection A work solution**: Take 1µL of 600x Detection A into 599 µL assay buffer before use it. Using this ratio to prepare Detection A working solution based on the detections.
3. **Detection B work solution**: Take1µL of 300x Detection B + 299 µL assay buffer before use it. Using this ratio to prepare Detection A working solution based on the detections.

Human Menin Standard Preparation:

4. Label test tubes as #1 through #8. Pipet 452 µL of 1x Assay Diluent into tube #1, and 400 µL into tubes #2 to #8 **as Fig.2 diagram below.**
5. Add 6 µL of the Human Menin Standard stock solution (10µg/mL) to tube #1 (131220pg/mL) and mix.
6. Make 3x serial dilutions of the standard using the 131220 pg/mL standard solution from tube #1 through #7 with sequential transfer of 200 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 131220, 43740, 14580, 4860, 1620, 540 and 180 pg/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 (µL)	452	400	400	400	400	400	400	400
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol (µL)	6	200	200	200	200	200	200	0
Final Conc (pg/ml)	131220	43740	14580	4860	1620	540	180	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 100 µL of standard, sample, or control per well, cover with the adhesive sealer. Incubate at **37°C for 1.5 hours.**
2. Aspirate each well, wash 3 times by filling each well with 200 µL Wash Buffer, each washing sits for 1 minute. Invert the plate and blot it against clean paper towels.
3. Add 100 µL of **Detection A work solution** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at RT **for 60 min.**
4. Aspirate each well and wash 3 times as Step2. After washing, invert the plate and blot it against clean paper towels.
5. Add 100 µL of **Detection B work solution** to each well. Incubate at RT **for 60 min.**

6. Aspirate each well, and wash for 3 times as Step 2 (*Note: Complete removal of residue liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Each washing sits for 1 minute. Invert the plate and blot it against clean paper towels.
7. Add 100 µL 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 50 µL 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.

CALCULATION OF RESULTS

Average the OD readings for each standard, control, and sample. Then subtract from 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. 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 100 pg/ml.

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

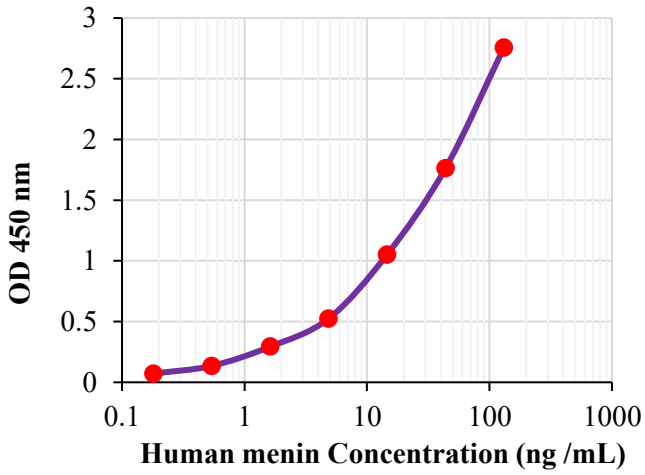
SPECIFICITY

This assay recognizes natural and recombinant human Menin.

RELATIVE PRODUCTS

Human IL-1ß 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- ß1 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



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