Human Phospho-Histone H2AX (S139) / Gamma-H2AX ELISA

For the quantitative determination of human γ-H2AX concentrations in cell culture supernates, serum, and plasma.

INTRODUCTION

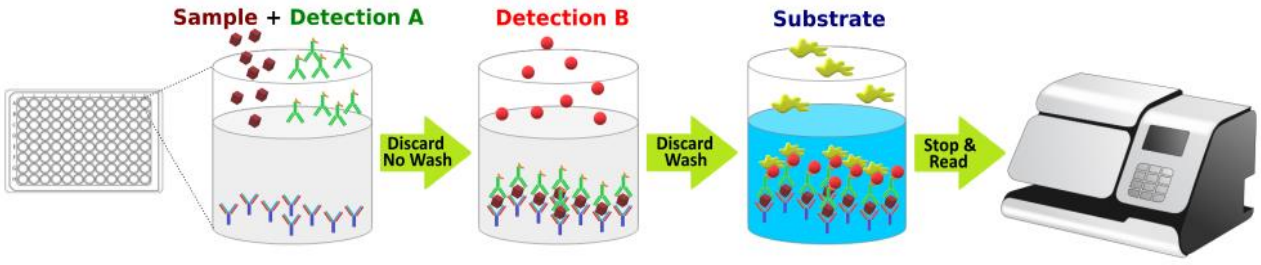
H2AX (H2A.X Variant Histone) is a Protein Coding gene, encoding a replication-independent histone that is a member of the histone H2A family, and generating two transcripts through the use of the conserved stem-loop termination motif, and the polyA addition motif. It is associated with diseases including Nijmegen Breakage Syndrome and Ataxia-Telangiectasia. In eukaryotes, DNA double strand breaks (DSBs) have been shown to trigger the phosphorylation of serine 139 at the carboxy terminus of histone H2AX resulting in gamma-H2AX (γ-H2AX).

Tribioscience's Fast Human γ-H2AX ELISA is designed to quantitatively detect human γ-H2AX levels in serum, plasma, and other biological samples. The main feature is that the kit uses our novel proprietary approaches to combine samples and detections into a one-step instead of the complicated traditional methods. It makes the assay simple, easy, accurate and fast. The Hands-on time can be within 2 hours, not need 4-5 hours (Fig. 1). The detection range is from 312.5 to 20000 pg/mL. The levels of human γ-H2AX samples are parallel to the standard curves obtained using the kit standards linearly. Therefore, the kit can be used to determine relative mass values for natural human γ-H2AX protein.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique (See Fig. 1). A monoclonal antibody specific for human γ-H2AX was pre-coated onto a microplate. Standards or samples and Detection Antibody are pipetted into the wells, and concurrently incubated for 2 hours. Then, just aspirate each well, no wash, directly add Streptavidin-HRP, incubate the complex. Following a wash to remove any unbound antibody and samples, an **ultra-sensitive TMB substrate solution** is added to the wells for color develops. The color intensity is in proportion to the amount of bound in the initial step. The intensity of the color is measured by plate read at 450 nm.

Fig. 1



KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Human γ-H2AX Microplate	TBS3265A	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human γ-H2AX.	Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
Human γ-H2AX Standard	TBS3265B	50 µl of Recombinant human γ-H2AX (200 ng/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS3265C	2.1 ml of biotin- human γ-H2AX antibody.	May be stored for up to 3 months at 2-8 °C.
Detection B	TBS3265D	12 ml of streptavidin HRP.	
Assay Diluent	TBS3265E	12 ml of a buffered protein base with preservatives.	
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 ℃. 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

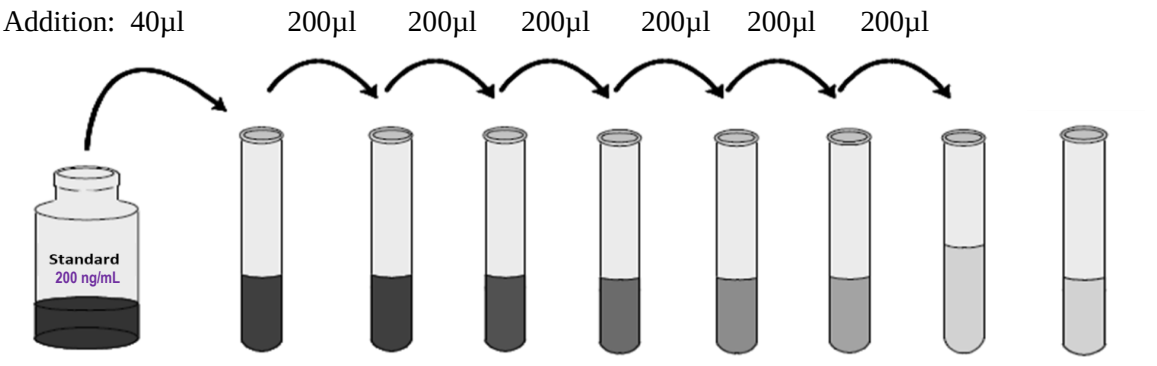
Bring all reagents to room temperature before use.

Wash Buffer: Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 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 γ-H2AX Standard Preparation: Label test tubes as #1 through #8. Pipet 360 µL of 1x Assay Diluent into tube #1, and 200 µL into tubes #2 to #8 **as diagram below.**

1. Add 40 µL of the Human γ-H2AX Standard stock solution (200 ng/mL) to tube #1 and mix.
2. Make 2x serial dilutions of the standard using the Tube#1 (20000 pg/mL standard solution) from Tube #2 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 20000, 10000, 5000, 2500, 1250, 625 and 312.5 pg/mL. Tube# 8 is Standard 0.

Fig.2 Diagram for Human γ-H2AX standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	360	200	200	200	200	200	200	200
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	40	200	200	200	200	200	200	0
Final Conc (pg/mL)	20000	10000	5000	2500	1250	625	312.5	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 µL of standard, sample, or control per well.
2. Add 20 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 2 hours**.
3. Aspirate each well (no wash). Invert the plate and blot it against clean paper towels.
4. Add 100 µL of **Detection B** to each well. Incubate at **RT for 1 hour**.
5. Aspirate each well, and wash for 3 times by filling each well with 300 µL 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.
6. Add 100 µL of **TMB Substrate** to each well. Incubate **at RT for 10-20min** (*Protect from light*). The color becomes blue.
7. 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).

8. Determine the optical density of each well within 20 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 542 nm or 570 nm. If wavelength correction is not available, subtract readings at 542 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 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 for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 3 is an example of typical Data.

SENSITIVITY

The minimum detectable dose (MOD) of human is typically 10 pg/ml.

The Intra-assay CV and the Inter-assay CV are <10%.

SPECIFICITY

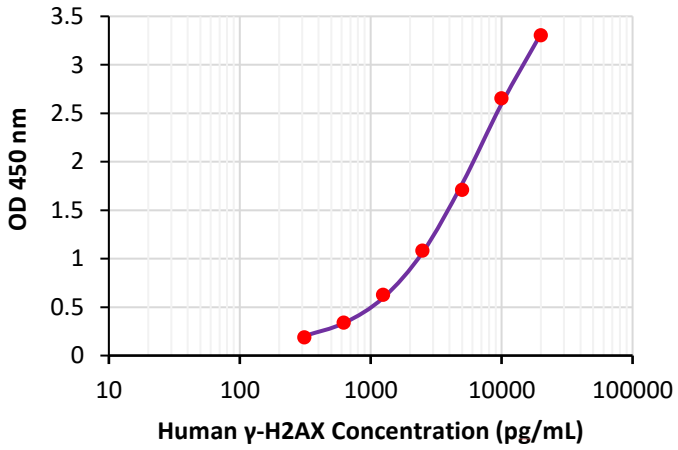
This assay specifically recognizes Histone H2AX phosphorylated at S139.

No cross-reactivity with unphosphorylated recombinant human (rh) Histone H2AX, rhHistone Macro H2A1, and rhHistone Macro H2A2.

RELATIVE PRODUCTS

Human p-Tau-181 ELISA (TBS3294)
Human Total H2AX ELISA (TBS3266)
Human Thr231 (p-T231) ELISA (TBS3295)
Human Thr217 (p-T217) ELISA (TBS3293)
Human IL-4 ELISA (TBS3221)
Human IL-6 ELISA (TBS3223)
Human IL-7 ELISA (TBS3224)
Human IL-8 ELISA (TBS3225)
Human IL-10 ELISA (TBS3226)
Human IL-13 ELISA (TBS3227)
Human IL-17 ELISA (TBS3228)
Human IL-22 ELISA (TBS3229)
Human IL-33 ELISA (TBS4245)
Human IFN-gamma ELISA (TBS3230)
Human TGF- ß1 ELISA (TBS3232)
Human GM-CSF ELISA (TBS3233)
Human MIP-1α ELISA (TBS3234)
Protein Cell Lysis Buffer (catalog# TBS5001)
Protein Assay Kit (Catalog# TBS2005)
TMB Substrate System (Catalog#TBS5021)

Fig. 3 Human γ-H2AX Standard Curve



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