Human Total GAPDH ELISA

For the development of sandwich ELISA tests to measure the human GAPDH in cell lysates.

INTRODUCTION

GAPDH (Glyceraldehyde-3-phosphate Dehydrogenase) is an enzyme that is involved in the second phase of glycolysis, a process that breaks down glucose and ultimately produces pyruvate for the trichloroacetic acid cycle (TCA cycle), and adenosine triphosphate (ATP), which is the primary energy source for important biological functions.

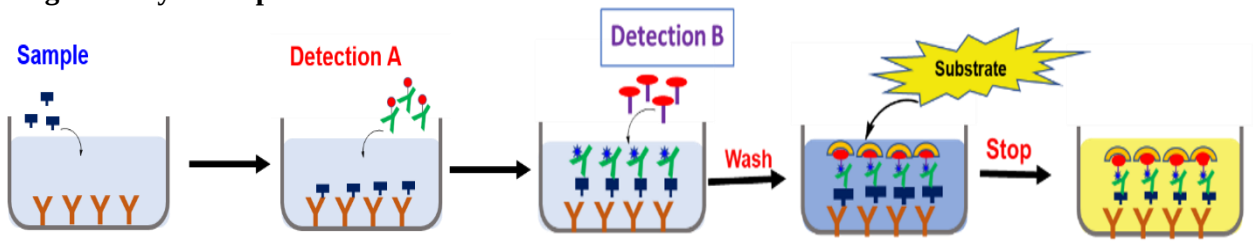
GAPDH is generally thought to be present at a constant level within the cell. In most cases, this stays true regardless of experimental treatment or technical procedures. Because of its ability to remain present, the measurement of GAPDH is used as an internal control for experimental error. GAPDH is widely used in different assay methods to investigate certain aspects of cell activity.

The Human Total GAPDH ELISA Kit is used to measure the presence of human GAPDH in cell lysates.

PRINCIPLE OF THE ASSAY

This assay employs our novel proprietary sandwich enzyme immunoassay techniques (See Fig. 1). an immobilized capture antibody specific for GAPDH 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 GAPDH bound in the initial step. The intensity of the color is measured by plate read at 450 nm.

Fig.1: Assay Principle



KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Human Total GAPDH Microplate	TBS32102A	96 well microplate (12 strips of 8 wells) coated with a Capture Antibody specific for human GAPDH.	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 GAPDH Standard	TBS32102B	100 µL of Recombinant human GAPDH protein (200 ng/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost the freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS32102C	12 mL of human biotinylated GAPDH antibody.	May be stored for up to 3 months at 2-8 °C.
Detection B	TBS32102D	12 mL of Streptavidin-HRP	
Assay Diluent	TBS32102E	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 at least two 96 well plates.

PRECAUTIONS

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

REAGENT PREPARATION

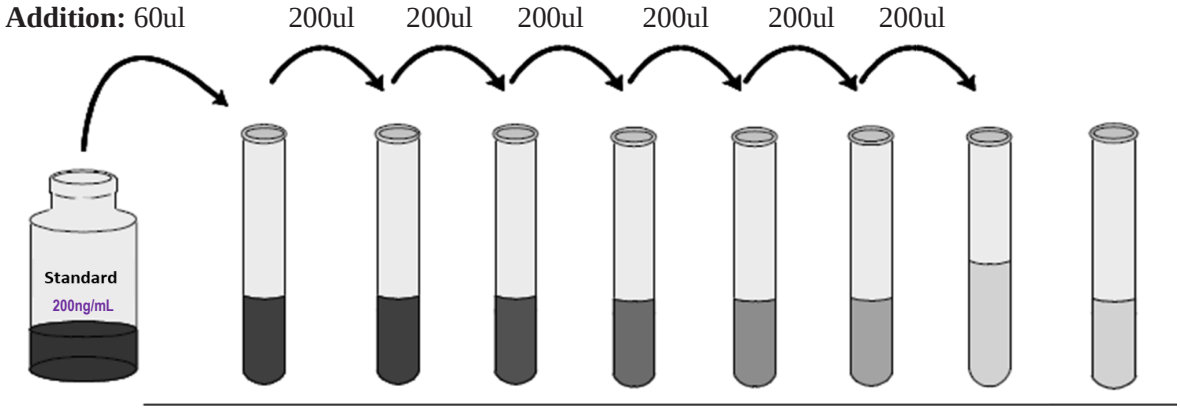
Bring all reagents to room temperature before use.

Wash Buffer: Add 1 part of Wash Solution Concentrate (10x) to 9 parts of deionized distilled water to prepare Wash Solution (1x) (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.*).

Human GAPDH Standard Preparation:

1. Label test tubes as #1 through #8. Pipet 420 µL of 1x Assay Diluent into tube #1, and 300 µL into tubes #2 to #8 **as diagram below.**
2. Add 60 µL of the Human Menin Standard stock solution (200 ng/mL) by dilution of 50X to tube #1 and mix.
3. Make 2.5x serial dilutions of the standard using the 25 ng/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 25, 10, 4, 1.6, 0.64, 0.256 and 0.1024 ng/mL. Tube# 8 is Standard 0.

Fig.2 Diagram for Human Total GAPDH Standard Preparation



Standard	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	420	300	300	300	300	300	300	300
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	60	200	200	200	200	200	200	0
Final Conc (ng/ml)	25	10	4	1.6	0.64	0.256	0.1024	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 **RT for 2 hours.**
2. Aspirate each well (***no wash***). Invert the plate and blot it against clean paper towels.
3. Add 100 µL of **Detection A** 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 (***no wash***). Invert the plate and blot it against clean paper towels.
5. Add 100 µL of **Detection B** to each well. Incubate at **RT for 30 min.**
6. 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.
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. 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

A standard curve is obtained by graphing the percentage absorbance of the standards (y-axis) versus their corresponding concentration (x-axis) on semi-log graph paper (which should be a linear relationship). A sample concentration can be read from this standard curve. Alternatively, total GAPDH concentration in the samples can be calculated with the regression equation correlating percentage absorbance to total GAPDH concentration. A graphing software can also be used for the quick analysis of the large number of samples.

TYPICAL DATA

This standard curve is provided for demonstration only as Fig.3. A standard curve should be generated for each set of samples assayed.

SENSITIVITY

The limit of detectable dose (LOD) of GAPDH is 0.15625ng/ml.

SPECIFICITY

This assay measures the total human/mouse/rat GAPDH in cell lysates.

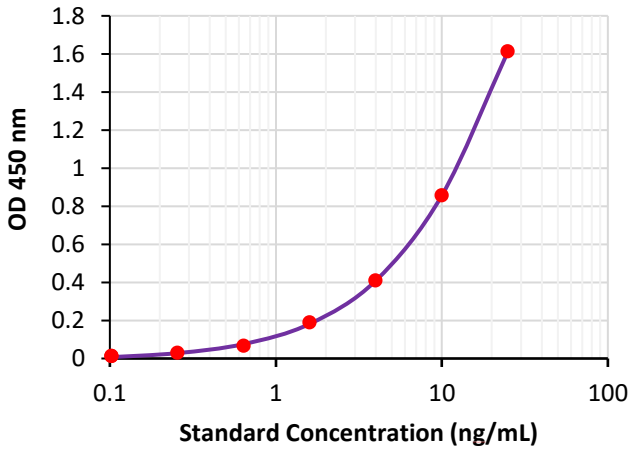
REFERENCES

1. Billiau, A.and P.Matthys (2009): Cytokine Growth Factor Rev.20:97.
2. Schoenborn,J.R and C.B. Wilson (2007): Adv.lmmunol. 96:41.
3. Pestka,S. et al. (2004): lmmunol. Rev.202:8.
4. Kelchtermans,H. et al. (2008): Trends lmmunol. 29:479.
5. Mclaren,J.E. and D.P. Ramji (2009): Cytokine Growth Factor Rev.20:125.

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-10 ELISA (TBS3226)
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)
Protein Cell Lysis Buffer (catalog# TBS5001)
Protein Assay Kit (Catalog# TBS2005)
TMB Substrate System (Catalog#TBS5021)

Fig.3: Total GAPDH Standard Curve



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