GAPDH Chemi-Luminescent ELISA Kit (Human) (OKCD03500)

Instructions for use

For the quantitative measurement of GAPDH in tissue homogenates, cell lysates, cell culture supernates and other biological fluids.

This product is intended for research use only.

Lot to lot kit variations can occur. Refer to the manual which has been provided with the kit.

Contents

1. Background

Principle

Aviva Systems Biology GAPDH Chemi-Luminescent ELISA Kit (Human) (OKCD03500) is based on standard sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific for GAPDH has been pre-coated onto a 96-wellplate (12 x 8 Well Strips). Standards or test samples are added to the wells, incubated and removed. A biotinylated detector antibody specific for GAPDH is added, incubated and followed by washing. Avidin-Peroxidase Conjugate is then added, incubated and unbound conjugate is washed away. An enzymatic reaction is produced through the addition of a luminol substrate which is catalyzed by the HRP to produce light emission. The light emission is read by a luminometer (or photo-multiplier equipped instrument) and the intensity of the emitted light is proportional to the amount of sample GAPDH captured in well.

Background

Has both glyceraldehyde-3-phosphate dehydrogenase and nitrosylase activities, thereby playing a role in glycolysis and nuclear functions, respectively. Participates in nuclear events including transcription, RNA transport, DNA replication and apoptosis. Nuclear functions are probably due to the nitrosylase activity that mediates cysteine S-nitrosylation of nuclear target proteins such as SIRT1, HDAC2 and PRKDC. Modulates the organization and assembly of the cytoskeleton. Facilitates the CHP1-dependent microtubule and membrane associations through its ability to stimulate the binding of CHP1 to microtubules . Glyceraldehyde-3-phosphate dehydrogenase is a key enzyme in glycolysis that catalyzes the first step of the pathway by converting D-glyceraldehyde 3-phosphate (G3P) into 3-phospho-D-glyceroyl phosphate. Component of the GAIT (gamma interferon-activated inhibitor of translation) complex which mediates interferon-gamma-induced transcript-selective translation inhibition in inflammation processes. Upon interferon-gamma treatment assembles into the GAIT complex which binds to stem loop-containing GAIT elements in the 3'-UTR of diverse inflammatory mRNAs (such as ceruplasmin) and suppresses their translation.

General Specifications

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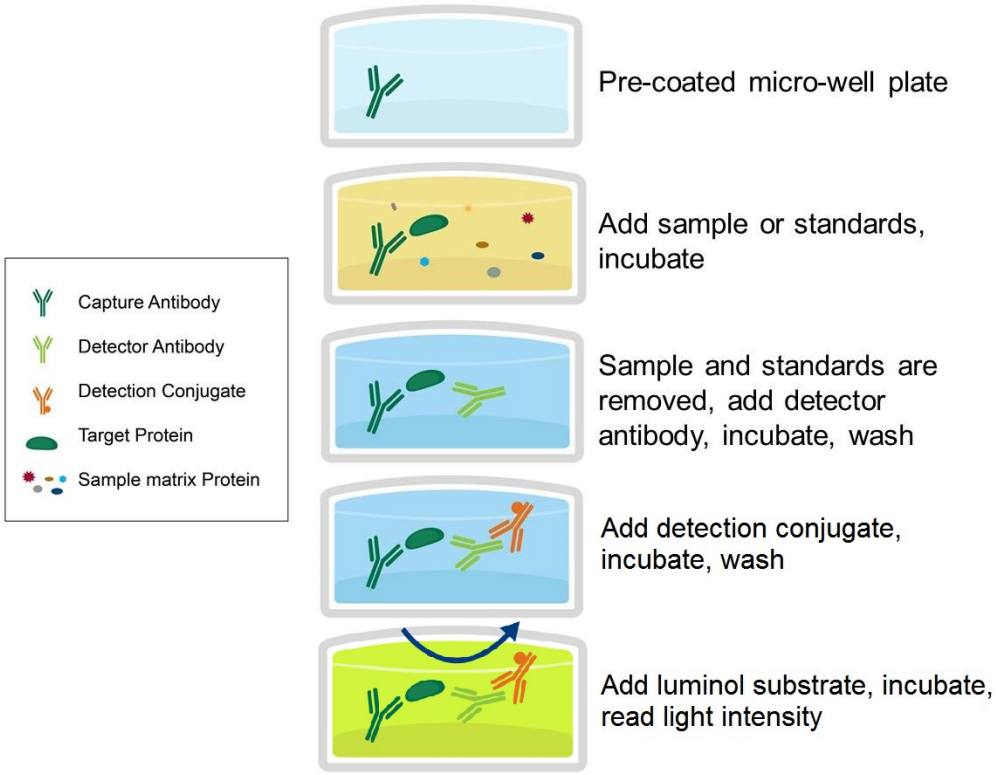
Range	0.27 - 200 ng/mL
LOD	< 0.12 ng/mL (Derived by linear regression of OD_{450} of the Mean Blank + 2xSD)
Specificity	Human Glyceraldehyde-3-phosphate dehydrogenase UniProt ID: P04406 GeneID: 2597 Target Alias: CDABP0047, G3PD, GAPD, Glyceraldehyde-3-phosphate dehydrogenase, MGC88685, OK/SW-cl.12, Peptidyl-cysteine S-nitrosylase GAPDH
Cross-Reactivity	No detectable cross-reactivity with other relevant proteins

2. Assay Summary



3. Storage and Stability

• Upon receipt store kit at 4°C for 1 month or -20°C for 6 months, noted exceptions below. Do not use past expiration date.

4. Kit Components

•The following reagents are the provided contents of the kit.

Description	Quantity	Storage Conditions
Anti-GAPDH Microplate	96 Wells (12 x 8 Well strips)	4°C for 1 Month or -20°C for 6 Months
GAPDH Lyophilized Standard	2 x 200 ng	
100X Biotinylated GAPDH Detector Antibody	1 x 120 µL	
100X Avidin-HRP Conjugate	1 x 120 µL	
Standard Diluent	1 x 20 mL	4°C for 6 Month
Detector Antibody Diluent	1 x 12 mL	
Conjugate Diluent	1 x 12 mL	
30X Wash Buffer	1 x 20 mL	
100X Luminol Substrate	1 x 2 mL	
Substrate Diluent	1 x 20 mL	

5. Precautions

• Read instructions fully prior to beginning use of the assay kit.

• Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.

• Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.

• For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).

• Kit cannot be used beyond the expiration date on the label.

6. Required Materials Not Supplied

• Luminometer or photo-multiplier tube (PMT) equipped microplate reader capable of the following parameters: lag time 30.0 seconds, read time 1.0 seconds per well.

• Automated plate washer (optional).

• Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.

• Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.

• New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.

• Absorbent paper or paper toweling.

• Distilled or deionized ultrapure water.

• 37°C Incubator (optional)

7. Technical Application Tips

• Do not mix or substitute components from other kits.

• To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.

• Samples exhibiting light intensity measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.

• Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.

• Replicate wells are recommended for standards and samples.

• Cover microplate while incubating to prevent evaporation.

• Do not allow the microplate wells dry at any point during the assay procedure.

• Do not reuse tips or tube to prevent cross contamination.

• Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.

• Completely remove of all liquids when washing to prevent cross contamination.

• Prepare reagents immediately prior to use and do not store, with the exception of the top standard.

• Equilibrate all materials to ambient room temperature prior to use (standards exception).

• For optimal results for inter- and intra-assay consistency, equilibrate all materials to 37°C prior to performing assay (standards exception) and perform all incubations at 37°C.

• Pipetting less than 1 µL is not recommended for optimal assay accuracy.

• Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.

• Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.

• Samples containing precipitates, fibrin strands or bilirubin, or are hemolytic or lipemic might cause inaccurate results due to interfering factors.

• Luminol Substrate is easily contaminated and labile. Handle carefully and protect from light.