GAPDH Activity Assay Kit

ARG83558 GAPDH Activity Assay Kit can be used to measure GAPDH in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, other biological fluids

Catalog number: ARG83558

Package: 100 tests

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PRINCIPLE OF THE ASSAY

ARG83558 GAPDH Activity Assay Kit provides a simple and sensitive method for monitoring GAPDH activity in various samples. In this assay, GAPDH catalyzes conversion of GAP into BPG and an intermediate, which reacts with a developer to form a colored product that absorbs maximally at 492 nm.

MATERIALS PROVIDED & STORAGE INFORMATION

Store Standard, Positive Control, Coenzyme and Substrate at -20 °C, all other component at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 vial (lyophilized)	-20 °C
Positive Control	1 vial (lyophilized)	-20 °C
Assay Buffer	4 x 30 ml	4 °C
Coenzyme	1 vial (lyophilized)	-20 °C
Substrate	30 µl x	-20 °C
Reaction Buffer	10 ml	4 °C
Dye Reagent A	1 vial (lyophilized)	4 °C
Dye Reagent B	1 ml	4 °C
Plate sealer	3 adhesive strips	

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 492 nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store Standard, Positive Control, Coenzyme and Substrate at -20 °C, all other component at 2-8°C. Use the kit before expiration date.Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell and Bacteria-Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation. Mix and sonicate with 1 ml Assay buffer per 5×10^6 cell or bacteria. Centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Tissue- Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **Substrate:** Reconstitute the **Substrate** with **1 ml** of Reaction Buffer. Allow the **Substrate** keep on bench for few minutes. Make sure the **Substrate** is dissolved completely and mixed thoroughly before use.
  The diluted Substrate is stable for 4 weeks at-20°C.
- **Coenzyme:** Reconstitute the **Coenzyme** with **1 ml** of Reaction Buffer. Allow the **Coenzyme** keep on bench for few minutes. Make sure the **Coenzyme** is dissolved completely and mixed thoroughly before use.
  The diluted Coenzyme is stable for 4 weeks at -80°C.
- **Dye Reagent A:** Reconstitute the **Dye Reagent A** with **9 ml** of distilled water. Allow the **Dye Reagent A** keep on bench for few minutes. Make sure the **Dye Reagent A** is dissolved completely and mixed thoroughly before use.
  The diluted Dye Reagent A is stable for 4 weeks at -20°C.
- **Standard:** Reconstitute the **Standard** with **1 ml** of distilled water, mix well, allow the **Standard** keep on bench for few minutes. Make sure the **Standard** is dissolved completely and mixed thoroughly before use.
  Then add 0.2 ml into 0.8 ml distilled water to yield 400 nmol/ mL standard. Perform 2-fold serial dilutions of the top standards to make the standard curve. The diluted **Standard** is stable for 4 weeks at -80°C.
- **Positive Control:** Reconstitute the **Positive Control** with **200 µl** of Assay Buffer. Allow the **Positive Control** keep on bench for few minutes. Make sure the **Positive Control** is dissolved completely and mixed thoroughly before use. The diluted **Positive Control** is stable for 4 weeks at -80°C.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Add 70 µl Reaction Buffer into each **Sample**, **Control** and **Positive Control** wells.
2. Add 10 µl Coenzyme into **Sample**, **Control** and **Positive Control** wells.
3. Add 10 µl Substrate into **Sample**, **Control** and **Positive Control** wells.
4. Add 10 µl Sample, Positive Control into **respective wells**.
5. Add 100 µl Standard into **Standard wells**.
6. Add 10 µl Distilled water into each **Control** wells.
7. Add 100 µl Distilled water into each **Blank** wells.
8. Add 90 µl Dye Reagent A into all wells.
9. Add 90 µl Dye Reagent B into all wells.
10. **Mix well. Incubate at RT for 5 min. Read the OD at 492nm.**

Summary of GAPDH Activity Assay Kit Procedure

Reagent	Sample	Control	Standard	Blank	Positive Control
Reaction Buffer	70 µl	70 µl	-	-	70 µl
Coenzyme	10 µl	10 µl	-	-	10 µl
Substrate	10 µl	10 µl	-	-	10 µl
Sample	10 µl		-	-	-
Positive Control	-	-	-	-	10 µl
Standard	-	-	100 µl	-	-
Distilled water	-	10 µl	-	100 µl	-
Dye Reagent A	90 µl	90 µl	90 µl	90 µl	90 µl
Dye Reagent B	10 µl	10 µl	10 µl	10 µl	10 µl
Mix well Incubate for 5 min at 37 °C Read the OD at 492 nm					

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of samples, standard and blank.

a.) Definition: one unit of GAPDH activity is defined as the enzyme oxidize 1 µmol NADH per minute.

$C_{Standard:}$ the standard concentration, 400 µmol/L = 0.4 µmol/ml;

$V_{Standard}$: the volume of standard, 100 µl = 0.1 ml;

CProtein: the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 5 minutes.

b.) Calculation:

Formula:

a). According to the protein concentration

GAPDH (U/mg) = = $(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times (V_{Sample} \times C_{Protein}) \times T]$

$= 0.8 \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times C_{Protein}]$

b). According to the weight

GAPDH (U/g) = = ($C_{Standard}$ × $V_{Standard}$) × (OD_{Sample} - $OD_{Control}$) / [($OD_{Standard}$ - OD_{Blank}) x (V_{Sample} × W / V_{assay}) × T]

= 0.8 × (OD_{Sample} - $OD_{Control}$) / [($OD_{Standard}$ - OD_{Blank}) x W]

c). According to the quantity of cell or bacteria

GAPDH ($U/10^4$) = ($C_{Standard}$ × $V_{Standard}$) × (OD_{Sample} - $OD_{Control}$) / [($OD_{Standard}$ - OD_{Blank}) x (V_{Sample} × N / V_{assay}) × T]

= 0.8 × (OD_{Sample} - $OD_{Control}$) / [($OD_{Standard}$ - OD_{Blank}) x N]

4. According to the volume

GAPDH (U/ml) = ($C_{Standard}$ × $V_{Standard}$) × (OD_{Sample} - $OD_{Control}$) / [($OD_{Standard}$ - OD_{Blank}) x V_{Sample} × T]

= 0.8 × (OD_{Sample} - $OD_{Control}$) / ($OD_{Standard}$ - OD_{Blank})