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Product Information

High Sensitivity Dihydroxyacetone Phosphate Assay Kit

Catalog Number **MAK275**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Dihydroxyacetone phosphate (DHAP) is a metabolic intermediate involved in many pathways, including glycolysis, gluconeogenesis, glycerol metabolism, phosphatidic acid synthesis, fat metabolism, and the Calvin cycle. Fructose-1,6-diphosphate is converted to DHAP and glyceraldehyde-3-phosphate (GAP) by aldolase. Both DHAP and GAP serve as the intracellular triose phosphate pool. DHAP can be further converted into GAP by triose phosphate isomerase (TPI). In humans, TPI deficiency is a rare autosomal disease. It causes hemolytic anemia, neurological diseases, and even death due to blockage of the glycolytic pathway and accumulation of DHAP in erythrocytes.

The High Sensitivity Dihydroxyacetone Phosphate Assay Kit is suitable for measuring low levels of DHAP. In this assay, TPI converts DHAP to GAP producing a coupled enzyme reaction, which results in a fluorometric product (λ_{ex} = 535 nm/λ_{em} = 587 nm), proportional to the amount of dihydroxyacetone phosphate present. This assay kit is simple and sensitive with the capability to detect to 0.5 μM of dihydroxyacetone phosphate in a variety of samples.

This kit is suitable for use with serum, plasma, and other biological fluids, and tissue and cell culture samples.

Components

The kit is sufficient for 100 assays in 96 well plates.

DHAP Assay Buffer Catalog Number MAK275A	25 mL
High Sensitivity Probe, in DMSO Catalog Number MAK275B	0.4 mL
DHAP Enzyme Mix Catalog Number MAK275C	1 vl
DHAP Developer Catalog Number MAK275D	1 vl
DHAP Standard, Catalog Number MAK275E	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate. It is recommended to use white plates with clear bottoms for fluorescence assays.
- Fluorescence multiwell plate reader
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

DHAP Assay Buffer – Allow buffer to come to room temperature before use.

High Sensitivity Probe – Warm to room temperature to thaw the solution prior to use. Store at –20 °C. Upon thawing, the probe is ready-to-use as supplied.

DHAP Enzyme Mix and DHAP Developer- Reconstitute each vial with 220 μL of DHAP Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Keep on ice while in use. Use within 2 months of reconstitution.

DHAP Standard - Reconstitute in 100 µL of water to generate 100 mM (100 nmole/µL) DHAP Standard Solution. Mix well by pipetting. Store at –20 °C. Keep on ice while is use. Use within 2 months of reconstitution.

Storage/Stability
The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

DHAP Standards for Fluorometric Detection
Dilute 10 µL of the 100 mM DHAP Standard with 990 µL of DHAP Assay Buffer to prepare a 1 mM standard solution. Dilute further to 50 µM (50 pmole/µL) by adding 50 µL of 1 mM DHAP Standard Solution to 950 µL of DHAP Assay Buffer. Add 0, 2, 4, 6, 8, and 10 µL of the diluted 50 pmole/µL DHAP standard into a 96 well plate, generating 0 (blank), 100, 200, 300, 400, and 500 pmole/well standards. Add DHAP Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Fluorometric assays require 50 µL of sample for each reaction (well).

Serum and plasma samples can be assayed directly.

Tissue (10 mg) or cells (1×10^6) can be homogenized in 100 µL of ice-cold DHAP Assay Buffer for 10 minutes on ice. Centrifuge the samples at 10,000 × *g* for 5 minutes to remove insoluble material. Collect the supernatant.

Add 2–50 µL of samples into duplicate wells of a 96 well plate. Bring samples to a final volume of 50 µL with DHAP Assay Buffer.

Notes: For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the range of the standard curve.

Because enzymes in samples may interfere with the assay, samples should be deproteinized with a 10 kDa MWCO spin filter prior to addition to the reaction

NADH present in the sample can generate background. Prepare parallel sample well(s) as sample background control(s) by omitting the DHAP Enzyme Mix in the Reaction Mix. The Sample Background Control readings can then be subtracted from the sample readings.

Assay Reaction
1. Set up the Master Reaction Mix according to the scheme in Table 1. 50 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Samples and Standards	Sample Control
DHAP Assay Buffer	43 µL	45 µL
High Sensitivity Probe	3 µL	3 µL
DHAP Enzyme Mix	2 µL	–
DHAP Developer	2 µL	2 µL

2. Add 50 µL of the Master Reaction Mix to each sample and standard control well. If using a sample background control, add 50 µL of Sample Control Mix to sample control wells. Mix well using a horizontal shaker or by pipetting.

3. Incubate the plate for 60 minutes at 37 °C. Protect the plate from light during the incubation.

4. Measure fluorescence intensity (λ_{ex} = 535/ λ_{em} = 587 nm).

Results

Calculations

The background for the assay is the value obtained for the 0 (blank) DHAP Standard. Correct for the background by subtracting the blank standard value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate DHAP Standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the Sample Background Control value from the sample readings to obtain the corrected fluorescence measurement. Using the corrected measurement, determine the amount of DHAP present in the sample from the standard curve.

Concentration of Dihydroxyacetone Phosphate

$$S_a/S_v = C$$

S_a = Amount of DHAP in the unknown sample (pmole) from standard curve
S_v = Sample volume (μL) added into the wells
C = Concentration of DHAP in sample

Dihydroxyacetone Phosphate ($C_3H_7O_6P$) molecular weight: 170.06 g/mol

Sample Calculation

Amount of DHAP (S_a) = 258.4 pmole (from standard curve)
Sample volume (S_v) = 25 μL

Concentration of DHAP in sample:

$$258.4 \text{ pmole}/25 \text{ μL} = 10.34 \text{ pmole}/\text{μL}$$

$$10.34 \text{ pmole}/\text{μL} \times 342.3 \text{ pg/pmole} = 3539 \text{ pg}/\text{μL}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use white plates with clear bottoms.
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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