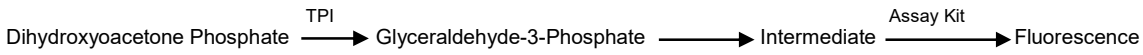
Dihydroxyacetone Phosphate Fluorometric Assay Kit (#BN00897)

(Catalog # BN00897; 100 assays; Store at -20°C)

I. Introduction:

Dihydroxyacetone Phosphate (DHAP) is an important intermediate in both lipid biosynthesis and glycolysis. In glycolysis, 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. Assay Genie's ihydroxyacetone Phosphate Assay kit is suitable for measuring low levels of DHAP typically found in a variety of samples. In this kit, TPI converts DHAP to GAP that undergoes a series of reaction and reduces to generate fluorescence. The fluorescence intensity generated is directly proportional to the amount of Dihydroxyacetone Phosphate. This simple, sensitive, and easy to use assay kit can detect Dihydroxyacetone Phosphate as low as 0.5 µM in a variety of samples.



II. Application:

- Measurement of Dhydroxyacetone Phosphate in various tissues/cells
- Analysis of metabolic pathways such as glycolysis, Calvin cycle in plants, methylglyoxal pathway and lipid biosynthesis
- Mechanistic study of human TPI deficiency

III. Sample Type:

- Animal tissues: e.g., liver, muscle etc.
- Cell culture: adherent or suspension cells
- Biological fluids: serum, plasma etc.

IV. Kit Contents:

Components	BN00897	Cap Code	Part Number
DHAP Assay Buffer	25 ml	WM	BN00897-1
Assay Kit (in DMSO)	0.4 ml	Blue	BN00897-2
DHAP Enzyme Mix	Lyophilized	Green	BN00897-3
DHAP Developer	Lyophilized	Red	BN00897-4
DHAP Standard	Lyophilized	Yellow	BN00897-5

V. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom. White plate is preferred for this assay.
- Multi-well spectrophotometer (ELISA reader)

VI. Storage Conditions and Reagent Preparation:

Store kit at –20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **DHAP Assay Buffer:** Bring to room temperature before use.
- **Assay Kit:** Ready to use as supplied. Warm to room temperature before use. Store at -20°C.
- **DHAP Enzyme Mix and DHAP Developer**: Reconstitute each vial with 220 µl DHAP Assay Buffer, making sure the material is completely dissolved. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Keep on ice while in use. Use within two months.
- **DHAP Standard:** Reconstitute with 100 µl dH_2O to generate 100 mM (100 nmol/µl) DHAP Standard solution. Store at -20°C. Keep on ice while in use. Use within two months.

VII. Dihydroxyacetone Phosphate Assay Protocol:

1. **Sample Preparation:** Serum or plasma samples can be measured directly. Tissue (10 mg) or cells (~10^6) should be rapidly homogenized with 100 µl ice cold DHAP Assay Buffer. Keep on ice for 10 min. Centrifuge at 10,000 X g for 5 min. and collect supernatant. Add 2-50 µl supernatant into a 96-well plate and bring the volume to 50 µl with DHAP Assay Buffer.

   Notes:

   a. Enzymes in sample may interfere with the assay. We suggest deproteinizing sample using Deproteinizing sample preparation kit or a 10 kDa cut-off spin filter to remove enzymes.

   b. For unknown samples, we suggest doing pilot experiment and testing several doses to ensure the readings are within the standard curve range.

   c. NADH in samples will generate background. For samples having NADH, prepare parallel sample well(s) as sample background control.

2. **Standard Curve Preparation:** Dilute 100 mM DHAP Standard to 1mM by adding 10 µl of 100 mM DHAP Standard to 990 µl Assay Buffer and mix. Dilute further to 50 µM (50 pmol/µl) by adding 50 µl of 1mM DHAP Standard to 950 µl Assay Buffer and mix well. Add 0, 2, 4, 6, 8, and 10 µl of diluted 50 pmol/µl DHAP Standard into a 96-well plate to generate 0, 100, 200, 300, 400, and 500 pmol/well Standard. Adjust the volume to 50 µl/well with DHAP Assay Buffer.
3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	*Background Control Mix
DHAP Assay Buffer	43 µl	45 µl
Assay Kit	3 µl	3 µl
DHAP Enzyme Mix	2 µl	----
DHAP Developer	2 µl	2 µl

Add 50 µl of Reaction Mix to each well containing Standards and test samples. Mix well. Incubate for 60 min. at 37°C, protected from light.

* For samples having NADH background, add 50 µl of Background Control Mix to sample background control well(s).

4. **Measurement:** Measure fluorescence (Ex/Em = 535/587 nm) in a micro plate reader.
5. **Calculation:** Subtract 0 DHAP Standard reading from all readings. Plot the Standard Curve. If sample background control reading is significant, subtract the background control reading from sample reading. Apply the corrected sample reading to the Standard Curve to get DHAP amount in the sample wells.

Sample Dihydroxyacetone Phosphate concentration (C) = B/V x Dilution Factor = pmol/µl = nmol/ml

Where: **B** = DHAP amount in sample well (pmol)
V = sample volume added into the reaction well (µl)

Dihydroxyacetone Phosphate ($C_3H_7O_6P$) molecular weight: 170.06 g/mol
Dihydroxyacetone Phosphate in sample can also be expressed in pmol/mg or mg/dL of sample.

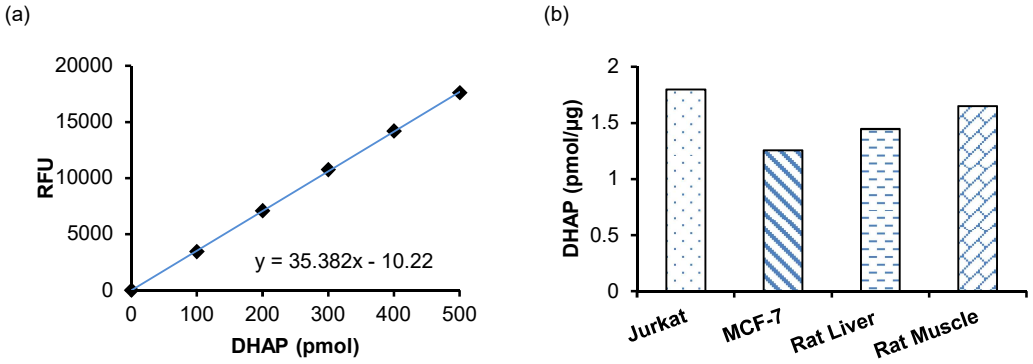


Figure: (a) Dihydroxyacetone Phosphate Standard Curve, (b) Measurement of Dihydroxyacetone Phosphate level in Jurkat (250 µg), and MCF-7 (150 µg) cell lysate and in rat liver (50 µg) and muscle (150 µg) lysate.

FOR RESEARCH USE ONLY! Not to be used on humans.