# PicoProbe™ 15-PGDH Activity Assay Kit (Fluorometric)

8/17

**(Catalog # K562-100; 100 assays; Store at -20°C)**

## I. Introduction:

15-Hydroxy prostaglandin dehydrogenase (15-PGDH; EC:1.1.1.141) is a rate-limiting enzyme for prostaglandin catabolism. It oxidizes prostaglandins (PG) to 15-keto metabolites. In general, 15-keto metabolites show reduced biological activity, when compared to PG. PGs are generated from arachidonic acid by cyclooxygenase (COX), and play an important role in inflammatory responses. Recent study found that 15-PGDH serves as a degrading enzyme for PG and serves as a tumor suppressor to inhibit cancer progression. Thus, study of 15-PGDH activity can elucidate and clarify unknown aspects related to cancer development and therapeutics. In BioVision's 15-PGDH Assay Kit, 15-PGDH oxidizes a substrate forming intermediates and NADH. The oxidation of NADH reduces a probe generating fluorescence at Ex/Em=535/587 nm. The activity of 15-PGDH is proportional to the fluorescent signal. BioVision's 15-PGDH Activity Assay Kit offers a rapid, simple, sensitive, and reliable test suitable for detecting as low as 1 µU of 15-PGDH activity.

## II. Application:

- Measurement of 15-PGDH activity in various tissues/cells.
- Analysis of prostaglandin catabolism in various cell types.

## III. Sample Type:

- Animal tissues: liver, kidney, lung etc.
- Cell culture: adherent or suspension cells

## IV. Kit Contents:

| Components | K562-100 | Cap Code | Part Number |
|---|---|---|---|
| 15-PGDH Assay Buffer | 25 ml | WM | K562-100-1 |
| PicoProbe™ (in DMSO) | 200 µl | Blue | K562-100-2 |
| 15-PGDH Developer | 1 vial | Red | K562-100-3 |
| 15-PGDH Substrate (in DMSO) | 100 µl | Purple | K562-100-4 |
| NADH Standard | 1 vial | Yellow | K562-100-5 |
| 15-PGDH Positive Control | 100 µl | Green | K562-100-6 |

## V. User Supplied Reagents and Equipment:

- 96-well white opaque plate with flat bottom.
- Multi-well spectrophotometer (fluorescence plate reader)
- Multi-channel pipette

## VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly spin small vials prior to opening. Read entire protocol before performing the assay. Unless specified, bring assay components to room temperature (RT) before use.

- **15-PGDH Developer:** Reconstitute with 220 µl of Assay Buffer. Avoid repeated freeze/thaw. Use within two months. Keep on ice while in use.
- **NADH Standard:** Reconstitute with 200 µl $dH_2O$ to generate 1 mM (1 nmol/µl) NADH Standard solution. Aliquot and store at –20°C. Use within two months. Keep on ice while in use.
- **15-PGDH Positive Control:** Aliquot and store at -20°C. Avoid repeated freeze/thaw. Use within two months. Keep on ice while in use.

## VII. 15-PGDH Activity Assay Protocol:

1. **Sample Preparation:** Homogenize tissue (~10 mg) or cells (1 x $10^6$) with 100 µl ice-cold 15-PGDH Assay Buffer. Keep on ice for 10 min. Centrifuge at 10,000 x *g*, 4°C for 5 min. and collect supernatant. Use ammonium sulfate precipitation method to remove small molecules that could interfere with the assay: Aliquot tissue samples (100 µl) to a clean centrifuge tube, and add 200 µl saturated (4.32 M) ammonium sulfate (BioVision Cat. # 7096) and place sample on ice for 30 min. Spin down samples at 10,000 x *g* at 4°C for 10 mins, discard the supernatant, and resuspend the pellet back to the original volume (100 µl) using 15-PGDH Assay Buffer. Add 2-50 µl into desired well(s) in a 96-well white plate. For 15-PGDH Positive Control, add 2-20 µl of 15-PGDH Positive Control into desired well(s). Adjust the volume of Positive Control and sample wells to 50 µl/well with 15-PGDH Assay Buffer.

**Notes:**

**a.** For unknown samples, we suggest doing pilot experiment and testing several amounts of sample to ensure the readings are within the Standard Curve range.

**b.** If sample has high background, prepare parallel sample well(s) as sample background control.

2. **NADH Standard Curve:** Dilute NADH Standard to 20 µM (20 pmol/µl) by adding 20 µl of 1 mM NADH Standard to 980 µl of 15-PGDH Assay Buffer. Add 0, 2, 4, 6, 8, and 10 µl of 20 µM NADH Standard into a series of wells in a 96-well plate to generate 0, 40, 80, 120, 160 and 200 pmol/well of NADH Standard. Adjust the volume to 50 µl/well with 15-PGDH Assay Buffer.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containin:

| | Reaction Mix | *Background Control Mix |
|---|---|---|
| 15-PGDH Assay Buffer | 46 µl | 47 µl |
| PicoProbe™ | 1 µl | 1 µl |
| 15-PGDH Developer | 2 µl | 2 µl |
| 15-PGDH Substrate | 1 µl | ---- |

Mix and add 50 µl of Reaction Mix into each well containing Standards, Positive Control, and Samples. Mix well.
* For samples having background, add 50 µl of Background Control Mix to sample background control well(s).

4. **Measurement:** Measure fluorescence (Ex/Em = 535/587 nm) immediately in kinetic mode for 10-40 min. at 37°C.
   Note: Incubation time depends on the 15-PGDH activity in the samples. We recommend measuring fluorescence in kinetic mode, and choosing two time points ($t_1$ and $t_2$) in the linear range to calculate the 15-PGDH activity of the samples. The NADH Standard Curve can be read in endpoint mode (i.e. at the end of incubation time).

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the NADH Standard curve. If sample background control reading is significant, subtract the sample background control reading from sample reading. Calculate the 15-PGDH activity of the test sample: $\Delta RFU = RFU_2 - RFU_1$. Apply ΔRFU to NADH Standard Curve to get B pmol of NADH generated by 15-PGDH during the reaction time ($\Delta t = t_2 - t_1$).

**Sample 15-PGDH Activity = B/(Δt X V) x D = pmol/min/µl = µU/µl = mU/ml**

Where: **B** is NADH amount in the sample well from Standard Curve (pmol)
**Δt** is reaction time (min.)
**V** is sample volume added into the reaction well (µl)
**D** is dilution factor (D=1 if undiluted)

15-PGDH Activity in samples can also be expressed in mU/mg of protein.

**Unit Definition:** One unit of 15-PGDH is the amount of enzyme that generates 1.0 µmol of NADH per min. at pH 7.2 at 37°C.

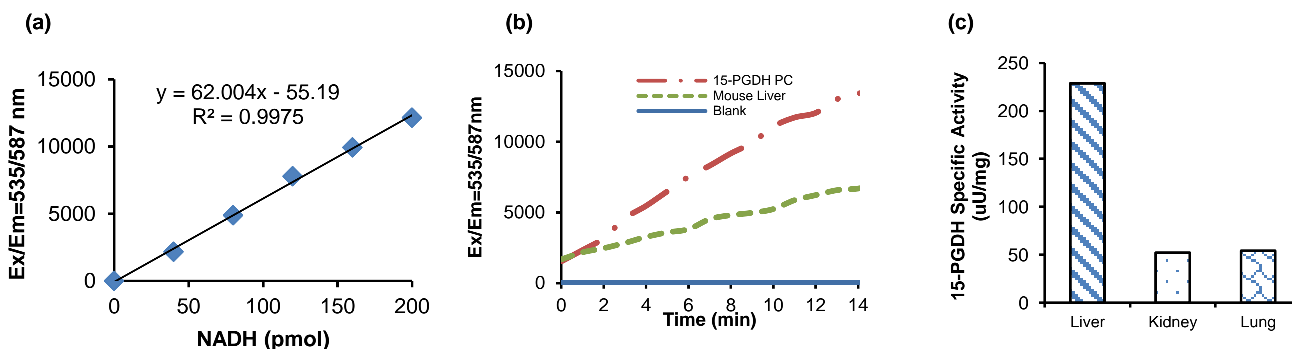


**Figure:** (a) NADH Standard Curve. (b) Kinetic measurement of 15-PGDH Specific Activity in lysates prepared from mouse liver (50 µg) and 15-PGDH Positive Control. (c) 15-PGDH specific activity of mouse lysates from liver (50 µg), kidney (16.5 µg) and lung (41 µg). Assays were performed following the kit protocol.

## VIII. RELATED PRODUCTS:

Cyclooxygenase (COX) Activity Assay Kit (Fluorometric) (K549)
Peroxidase Activity Assay Kit (K772)
Myeloperoxidase (MPO) Colorimetric Activity Assay Kit (K744)
Myeloperoxidase (MPO) Inhibitor Screening Kit (K746)
COX-2 Inhibitor Screening Kit (K547)
Myeloperoxidase (MPO) Peroxidation Activity Assay Kit (K747)
Myeloperoxidase (MPO) Fluorometric Activity Assay Kit (K745)
15-PDGH Inhibitor Screening Assay (K503-100)

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